

THE AMERICAN JOURNAL  
OF  
PHYSIOLOGY.

EDITED FOR

**The American Physiological Society**

BY

H. P. BOWDITCH, M.D., BOSTON

R. H. CHITTENDEN, Ph.D., NEW HAVEN

W. H. HOWELL, M.D., BALTIMORE

FREDERIC S. LEE, Ph.D., NEW YORK

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THE  
AMERICAN JOURNAL  
OF  
PHYSIOLOGY

VOLUME XXI.

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BOSTON, U.S.A.  
GINN AND COMPANY  
1908.

720  
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University Press  
JOHN WILSON AND SON, CAMBRIDGE, U.S.A.

# CONTENTS.

NO. I, FEBRUARY 1, 1908.

	PAGE
ON THE MECHANISM OF THE EMBRYONIC HEART RHYTHM IN LIMULUS. <i>By A. J. Carlson and W. J. Meek.</i> . . . . .	1
THE CONDUCTIVITY PRODUCED IN THE NON-CONDUCTING MYOCARDIUM OF LIMULUS BY SODIUM CHLORIDE IN ISOTONIC SOLUTION. <i>By</i> <i>A. J. Carlson</i> . . . . .	11
A NOTE ON THE REFRACTORY STATE OF THE NON-AUTOMATIC HEART MUSCLE OF LIMULUS. <i>By A. J. Carlson</i> . . . . .	19
THE RATE OF OXIDATION OF SUGARS IN AN ACID MEDIUM. <i>By H. H.</i> <i>Bunzel</i> . . . . .	23
PERFUSION EXPERIMENTS ON EXCISED KIDNEYS.—IX. THE EFFECTS OF VARIOUS POISONS. <i>By Torald Sollmann and R. A. Hatcher</i> . . . .	37
THE EFFECT OF VAGUS INHIBITION ON THE OUTPUT OF POTASSIUM FROM THE HEART. <i>By W. H. Howell and W. W. Duke</i> . . . .	51
CHEMICAL STUDIES ON GROWTH.—IV. THE TRANSFORMATION OF GLYCOGEN BY THE ENZYMES OF EMBRYONIC TISSUES. <i>By Lafayette</i> <i>B. Mendel and Tadasu Saiki</i> . . . . .	64
CHEMICAL STUDIES ON GROWTH.—V. THE AUTOLYSIS OF EMBRYONIC TISSUES. <i>By Lafayette B. Mendel and Charles S. Leavenworth</i> . . .	69
CHEMICAL STUDIES ON GROWTH.—VI. CHANGES IN THE PURINE, PENTOSE, AND CHOLESTEROL-CONTENT OF THE DEVELOPING EGG. <i>By Lafayette B. Mendel and Charles S. Leavenworth</i> . . . . .	77
CHEMICAL STUDIES ON GROWTH.—VII. THE CATALASE OF ANIMAL EMBRYONIC TISSUES. <i>By Lafayette B. Mendel and Charles S.</i> <i>Leavenworth</i> . . . . .	85
CHEMICAL STUDIES ON GROWTH.—VIII. THE OCCURRENCE OF LIPASE IN EMBRYONIC ANIMAL TISSUES. <i>By Lafayette B. Mendel and</i> <i>Charles S. Leavenworth</i> . . . . .	95

	PAGE
CHEMICAL STUDIES ON GROWTH.—IX. NOTES ON THE COMPOSITION OF EMBRYONIC MUSCULAR AND NERVOUS TISSUES. <i>By Lafayette B. Mendel and Charles S. Leavenworth</i> . . . . .	99
CHEMICAL STUDIES ON THE CELL AND ITS MEDIUM.—III. THE FUNCTION OF THE INORGANIC SALTS OF THE PROTOZOAN CELL AND ITS MEDIUM. <i>By Amos W. Peters</i> . . . . .	105
ACAPNIA AND SHOCK.—I. CARBON-DIOXID AS A FACTOR IN THE REGULATION OF THE HEART-RATE. <i>By Vandell Henderson</i> . . . .	126

## NO. II, MARCH 2, 1908.

THE PYRIMIDINE DERIVATIVES IN NUCLEIC ACID. <i>By Thomas B. Osborne and F. W. Heyl</i> . . . . .	157
THE INCREASE IN THE OSMOTIC CONCENTRATION OF THE BLOOD DURING ETHER AND CHLOROFORM ANÆSTHESIA. <i>By A. J. Carlson and A. B. Luckhardt</i> . . . . .	162
A NOTE ON THE UNION OF THE PROTEINS OF SERUM WITH ALKALI. <i>By Lawrence J. Henderson</i> . . . . .	169
CONCERNING THE RELATIONSHIP BETWEEN THE STRENGTH OF ACIDS AND THEIR CAPACITY TO PRESERVE NEUTRALITY. <i>By Lawrence J. Henderson</i> . . . . .	173
THE REACTION OF BLINDED LOBSTERS TO LIGHT. <i>By Philip B. Hadley</i> . . . . .	180
THE RELATION OF IONS TO CONTRACTILE PROCESSES.—II. THE RÔLE OF CALCIUM SALTS IN THE MECHANICAL INHIBITION OF THE CTENOPHORE SWIMMING-PLATE. <i>By R. S. Lillie</i> . . . . .	200
THE INFLUENCE OF VARIOUS LYMPHAGOGUES ON THE RELATIVE CONCENTRATION OF BACTERIO-AGGLUTININS IN SERUM AND LYMPH. <i>By B. Braude and A. J. Carlson</i> . . . . .	221
THE RELATIVE RESISTANCE OF THE HEART GANGLIA, THE INTRINSIC NERVE PLEXUS, AND THE HEART MUSCLE TO THE ACTION OF DRUGS. <i>By W. J. Meck</i> . . . . .	230
THE RELATIVE HEMOLYTIC POWER OF SERUM AND LYMPH UNDER VARYING CONDITIONS OF LYMPH FORMATION. <i>By W. T. Hughes and A. J. Carlson</i> . . . . .	236
THE COMPRESSIBILITIES OF GELATINE SOLUTIONS AND OF MUSCLE. <i>By Lawrence J. Henderson and F. N. Brink</i> . . . . .	248
THE SENSORY NERVES OF THE HEART AND BLOOD VESSELS AS A FACTOR IN DETERMINING THE ACTION OF DRUGS. <i>By D. E. Jackson and S. A. Matthews</i> . . . . .	255
ON A SERIES OF FEEDING AND INJECTION EXPERIMENTS FOLLOWING THE ESTABLISHMENT OF THE ECK FISTULA IN DOGS. <i>By P. B. Hawk</i> . . . . .	259

# Contents.

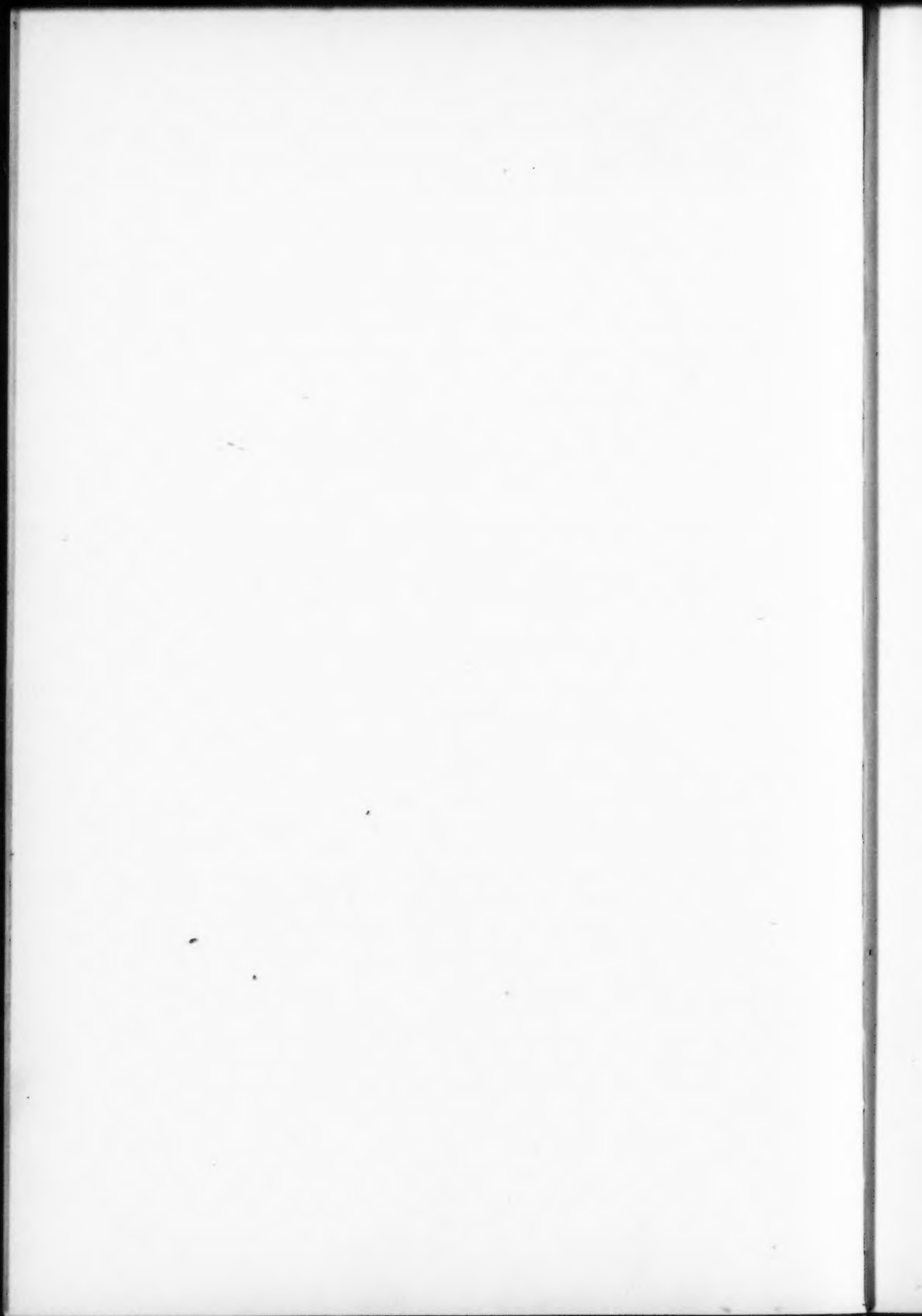
vii

NO. III, APRIL 1, 1908.

	PAGE
SOME EFFECTS OF CARDIAC NERVES UPON VENTRICULAR FIBRILLATION. <i>By Walter E. Garrey</i> . . . . .	283
GLUCOSE IN SALIVA. <i>By A. J. Carlson and J. G. Ryan</i> . . . . .	301
FURTHER STUDIES ON RESISTANCE TO LACK OF OXYGEN. <i>By Wales H. Packard</i> . . . . .	310
THE DIRECT UTILIZATION OF THE COMMON SUGARS BY THE TISSUES. <i>By Hugh McGuigan</i> . . . . .	334
ON GLYCOLYSIS. <i>By Hugh McGuigan</i> . . . . .	351
STUDIES IN RESUSCITATION.—II. THE REFLEX EXCITABILITY OF THE BRAIN AND SPINAL CORD AFTER CEREBRAL ANÆMIA. <i>By F. H. Pike, C. C. Guthrie, and G. N. Stewart</i> . . . . .	359

NO. IV, MAY 1, 1908.

DIRECT AND REFLEX RESPONSE OF THE CARDIO-INHIBITORY CENTRE TO INCREASED BLOOD PRESSURE. <i>By J. A. E. Eyster and D. R. Hooker</i> . . . . .	373
THE ANTAGONISTIC ACTION OF CALCIUM UPON THE INHIBITORY EFFECT OF MAGNESIUM. <i>By S. J. Meltzer and J. Auer</i> . . . . .	400
A STUDY OF THE EQUILIBRIUM BETWEEN CARBONIC ACID, SODIUM BICARBONATE, MONO-SODIUM PHOSPHATE, AND DI-SODIUM PHOSPHATE AT BODY TEMPERATURE. <i>By Lawrence J. Henderson and O. F. Black</i> . . . . .	420
THE THEORY OF NEUTRALITY REGULATION IN THE ANIMAL ORGANISM. <i>By Lawrence J. Henderson</i> . . . . .	427
THE ACTION OF STRONTIUM COMPARED WITH THAT OF CALCIUM AND MAGNESIUM. <i>By S. J. Meltzer and J. Auer</i> . . . . .	449
SOME VASOMOTOR CHANGES IN THE CEREBRAL VESSELS OBTAINED BY STIMULATING THE CAROTID PLEXUSES. <i>By Carl J. Wiggers</i> . . . . .	454
THE EFFECT OF HÆMORRHAGE UPON THE VASOMOTOR REFLEXES. <i>By W. T. Porter and H. K. Marks</i> . . . . .	460
INDEX . . . . .	467



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NO. 1.

ON THE MECHANISM OF THE EMBRYONIC  
HEART RHYTHM IN LIMULUS.

BY A. J. CARLSON AND W. J. MEEK.

[From the Marine Biological Laboratory, Woods Hole, and the Hull Physiological Laboratory, the University of Chicago.]

I. INTRODUCTORY.

SINCE the work of His, Jr., and of His, Jr. and Romberg<sup>1</sup> on the time of development of nervous tissue in the embryonic heart in relation to the time of the appearance of the heart activity, it is generally held that in the vertebrates the embryonic heart begins to beat before the "anlage" of the cardiac nervous system is in evidence. The work of His was mainly on fish and chick embryos, but there is some evidence that the embryonic heart of mammals exhibits the same conditions. These conclusions of His, Jr., with reference to the mechanism of the embryonic heart rhythm in the vertebrates falls back on the theory of Balfour that the autonomic and sympathetic nervous systems develop from neuroblasts that have migrated from the neural tube, and are therefore of epiblastic origin. This view appears to have the preponderance of the evidence in its favor and is generally accepted to-day. But it must still be classed as a theory, as it has not been demonstrated that all the ganglion cells in the sympathetic system and the cardiac and intestinal plexuses have that origin.

<sup>1</sup> HIS, JR., and ROMBERG: Fortschritte der Medizin, 1890, viii, p. 374; HIS, JR., Arbeiten aus dem medizinische Klinik zu Leipzig, 1893, i, p. 14.

In case all of the ganglion cells in the sympathetic ganglia and the cardiac and visceral plexuses are emigrants from the neural crests there is greater prospect of a decisive solution of the problem than there is in case this nervous tissue differentiates out of the mesoblastic tissue *in situ*, as held by Remak, Kupffer, Kölliker, and others.<sup>2</sup> If part of the cardiac plexus develops *in situ*, the question resolves itself into the determination at what stage the differentiation has gone far enough to allow the application of the term "nervous tissue," that is, when it begins to function. This cannot be settled by the microscope or by staining reactions, at least not with our present methods of microchemistry. But even if all the nerve cells in the heart are epiblastic in origin and have reached the heart by migrations along the path of the blood vessels, the difficulty of securing conclusive data is still great. Nerve fibres may reach the heart in advance of nerve cells. Embryonic nerve fibres can readily be distinguished from the surrounding tissues when a number of them lie close together in a more or less compact bundle, but may be readily overlooked or mistaken for developing connective tissue fibres when they pass singly through the mesoblast. Moreover, there is diversity of views as to what cells are and are not nerve cells, even in the adult heart. No one doubts that the large and often capsulated cells of the main ganglia in the sinus venosus, in the auricles, the auricular septum, and at the base of the ventricles are nerve cells. But the smaller cells distributed in the nerve net throughout the greater part of the myocardium and in the walls of the blood vessels are regarded by some as connective tissue cells, by others as nerve cells. The question is still open. In some instances at least these smaller non-capsulated cells on the course of the nerve net are true nerve cells, as their processes enter the nerve net.<sup>3</sup> If this is the situation as regards the nature of some of the heart cells of the adult heart, where the tissue differentiation has reached its maximum, it is evident that the question is at least not simpler or nearer solution in the embryonic heart. These smaller cells whose nature is in dispute may be nerve cells developed either from the mesoblast *in situ*, or emigrating neuroblasts that have not been distinguished from the cells of the hypoblast tissues. More conclusive work is therefore needed on the embryonic heart of vertebrates before this question is determined.

<sup>2</sup> PATTERSON: Philosophical transactions, 1890, clxxxi, p. 161.

<sup>3</sup> CARLSON: Archiv für die gesammte Physiologie, 1905, cix, p. 51.



While the peristalsis of the digestive tract is not myogenic, it is nevertheless still held by some physiologists that the smooth musculature of the digestive tract is automatic. Yanase<sup>4</sup> has recently shown that there is no basis for this view in the physiology of the embryo. In guinea-pig embryos the earliest movements of the alimentary canal appear about the fourth week and *pari passu* with the appearance of the Auerbach's plexus. It is obvious, however, that we cannot infer from this condition in the embryonic digestive tract that the same relations hold good for the embryonic heart. The movements of the digestive tract are no more useful or essential to the life of the embryo than the movements of the limbs, while the rhythm of the heart is a necessary factor. The latter may therefore, as it begins much earlier, be more primitive and simple in its mechanism than the former.

The theory that the embryonic heart of vertebrates begins to beat before any nervous tissue is developed in it or has reached it from the neural tube is generally construed as a fact in support of the myogenic theory of the nature of the heart rhythm. It is true that the heart activity begins before true myocardium has developed, but this contractile embryonic heart tissue develops into the heart muscle of the adult. If it shall finally be demonstrated that this embryonic myocardium is automatic in the total absence of nervous impulses, the most that can be claimed from that fact alone is that it throws the burden of proof on the rival theory of the heart rhythm. If the heart begins to beat in the total absence of nervous impulses, there seems to be no good reason why it should not continue to beat from the same cause during adult life. But it is also possible that with the development of the heart itself to a more complex organ, and with the differentiation of the contractile embryonic heart tissue into the muscular syncytium of the adult heart, the contractile tissue loses its primitive automatism, and that this is taken up by the cardiac ganglia and nerve plexus. This hypothesis may prove to be true, but it is so far without basis in fact or support in analogy. We know as yet of no organ in the animal body in which such transfer, as it were, of a fundamental property from one tissue to another takes place in development. The only *a priori* objection to the hypothesis that appears to us is this, that it is difficult to comprehend how this loss of automatism by the contractile heart tissue and its assumption by the cardiac nervous tissue can take place without

<sup>4</sup> YANASE: *Archiv für die gesammte Physiologie*, 1907, cxvii, p. 345.

production of inco-ordinations of the heart to an extent injurious to the embryo, assuming that the change takes place during embryonic life.

The neurogenic theory has been demonstrated for the adult heart of *Limulus*. The automatic ganglion giving rise to the heart rhythm is a compact nerve cord situated superficially to the myocardium on the dorsal side (Fig. 1). The literature contains no data on the time of development of this ganglion with reference to the beginning of the heart activity in the embryo. As the automatic ganglion



FIGURE 1.—Diagram of the cross section of the adult *Limulus* heart. *LN*, lateral nerve. *NC*, nerve cord or automatic ganglion.

is compact and invariably located in the median line on the dorsal side of the heart, we reasoned that it ought not to be difficult to determine the time of its appearance in the embryo. We undertook the work fully expecting to find this ganglion on the heart at the time the heart rhythm appears, but our results did not substantiate this expectation.

It appears that the heart rhythm in *Limulus* begins as a myogenic mechanism, so to say, although no fibrillary structure or transverse striations can be made out in the contractile tissue at the time the rhythm begins. In *Limulus* we have therefore a demonstration of the hypothesis of exchange of function of the myocardium and the local nervous tissue in development.

## II. METHODS.

Our material was obtained at Woods Hole, Mass., during June and July of last year. The breeding period of the king crab in that region is ordinarily completed at the end of May, but last year, owing to cold weather, it extended over the whole month of June and the first part of July. A breeding-place situated at a convenient distance from the laboratory was found. By examining the breeding-ground after each retreating high tide the new nests are readily discovered. A portion of the eggs from each nest was transferred to aquaria in the laboratory, and the nests were marked and numbered. Eggs from each nest, both from the lot remaining in the nest and from the lot in the laboratory aquaria, were then examined every day, the development of the heart followed, and its earliest rhythm noted.

Under the temperature conditions in the Woods Hole region during June of last year the rate of development was such that the heart first began to beat during the twenty-second or twenty-third day. There are some individual variations, but in almost every case the heart rhythm had begun before the end of the twenty-third day. The control lots kept in the aquaria developed a little slower. Of course, under other conditions of temperature the development may be either faster or slower. There is no difficulty in making out the outline of the developing heart through the transparent epidermis and egg membrane by means of the low-power microscope or a strong magnifying lens. The embryos may also be removed from the egg membrane without difficulty. In later stages the heart is obscured by the opacity of the greatly thickened epidermis dorsal to the heart.

Embryos of the stages from the nineteenth to the thirty-third day were fixed, and serial sections, transverse and longitudinal, prepared. The preparation of complete series of sections of *Limulus* embryos is rendered difficult by the great amount of brittle yolk inside the embryo. In cutting the sections this yolk tends to crumble and ruin the rest of the tissues. The fixing fluids tending to render the yolk part less brittle proved in our case to be Perenyi and Carnoy solutions.

Numerous attempts were made to remove the epidermis dorsal to the heart in the living embryos so as to get surface preparations of the dorsal wall, but without success. The dorsal side of the heart is so firmly attached to the epidermis by numerous connective tissue ligaments that we were unable to remove the latter without tearing the delicate heart walls to such an extent that the fragments could not be oriented with a certainty. Surface preparations of the dorsal heart wall would be an invaluable aid to the section method in determining the time of the appearance of the nerve-cord anlage.

The embryonic development of the *Limulus* heart has been described in detail by Kishinouye<sup>5</sup> and by Kingsley.<sup>6</sup> The heart is formed from the mesoblastic walls of the coelomic cavity. The walls of the coelomic cavity of each segment meet in the middle line and fuse in the dorsal and ventral lines, thus forming the heart tube. Fusion occurs also at the segmental junctions except for the space

<sup>5</sup> KISHINOUE: *Journal of College of Science, Imperial University of Japan*, 1893, v, p. 53.

<sup>6</sup> KINGSLEY: *Journal of morphology*, 1893, viii, p. 295.

of the lateral slits or ostia. The heart tube is formed earliest in the posterior region of the embryo, and the formation proceeds from thence anteriorly. Both Kingsley and Kishinouye describe and figure the embryonic heart up to the twenty-third day stage, that is, past the stage of the first appearance of the heart rhythm, but neither of these observers makes any mention of the presence of the heart ganglion.

### III. RESULTS.

1. At the stage of the beginning of the heart rhythm no nerve cord or ganglion on the dorsal side of the heart can be made out in



FIGURE 2. — Cross section (semi-diagrammatic) of the middle region of the embryonic *Limulus* heart at about the twenty-second day stage, showing a thin-walled tubular syncytium in which the nuclei are embedded. No indication of the nerve-cord anlage. *V*, ventral side of heart.



FIGURE 3. — Surface view of a portion of the embryonic heart wall at about the twenty-second day stage, showing the absence of distinct separation of the cells.

any of our preparations. Neither are the lateral nerves in evidence. The heart consists of a thin-walled tube. Kishinouye and Kingsley describe it as composed of a single layer of cells. In our preparations no distinct fibrillary structure or transverse striations can be made out. Moreover, there appears to be no distinct boundary between the cells. This is particularly evident in the flat preparations of the heart wall. The myocardium, or rather the tissue that develops into the myocardium, may therefore be described as a syncytium, even at this early stage. Semi-diagrammatic representations of the appearance of the heart in transverse section and surface view are given in Figs. 2 and 3.

It must not be understood, however, that the twenty-two or twenty-three day old embryonic heart looks in cross section as isolated and free from the adjacent tissues as indicated in Fig. 2. As a matter of fact, numerous cells adhere to the surface of the heart on all sides. Most of these cells are in progress of elongation to form the numerous suspensory ligaments of the heart. Blood cor-

puscles (white) also adhere to the heart wall at times, as the heart is in reality suspended in a blood sinus. There is no difficulty in distinguishing the blood corpuscles from the contractile tissue and from possible embryonic ganglion cells. But this is not the case with the connective tissue cells. Neither in their form nor in their staining reaction do some of these cells differ from some of the embryonic ganglion cells in the ventral nerve cord. We are therefore not able to affirm that no ganglion cells are present in any part of the twenty-two or twenty-three day old *Limulus* heart. Such cells may be there scattered among the connective tissue cells. But it is certain that there is no anlage of the nerve cord in the dorso-median line, nor can any nerve plexus or lateral nerve trunks be discovered.

The histogenesis of the cardiac nervous tissue in *Limulus* and other arthropods is, so far as we can learn, not worked out. Until this is done, and until we have developed microchemical methods that will enable us to distinguish with a certainty nerve cells and their processes from mesoblasts at all stages of embryonic development, this problem must remain in the same condition in the case of *Limulus* heart as it does in the case of the vertebrate heart. The *Limulus* heart and the vertebrate heart begin to beat before the anlage of the adult heart ganglion is in evidence, but our methods do not allow us to affirm that there is no embryonic nervous tissue there at all. In the case of the *Limulus* heart the relatively late appearance of the heart rhythm seems to point to the presence of nervous tissue in the heart at the time of the first contraction. But this relative lateness of the heart activity may be related to the fact that the yolk is situated in the centre of the embryo, and to the general slow development of the other tissues in this animal.

2. In none of our series were we able to discover the beginning of the nerve cord dorsal to the heart until the stage of twenty-eight to twenty-nine days. In that stage there appears, in the region that we may approximately describe as the middle third of the heart, a more or less uniform column of cells in the portion corresponding to that of the heart ganglion in the adult. These cells are mostly of the bipolar type, but their processes cannot be followed for any appreciable distance from the cell body. The dorsal nerve plexus and the lateral nerves cannot be made out even at this late stage, and the nerve cord itself has not yet extended to the anterior third and the posterior third of the heart. Carlson has shown that in the adult the nerve cord in the anterior region of the heart is composed

almost entirely of nerve fibres. In the posterior third of the heart the ganglion cells are distributed all through the nerve cord. The ganglion in the region of the middle third of the heart is the centre of greatest automatism. This part of the ganglion is the earliest to appear in the embryo. Evidently the ganglion cells proliferate from

this region towards the posterior and to some extent towards the anterior end of the heart. A series of cross sections of the heart at the twenty-nine day stage illustrating these points are given in Fig. 4.

At the twenty-eight to twenty-nine day stage the contractile heart tissue appears faintly cross-striated. But the heart walls do not seem to be much thicker than in the twenty-third day stage.

At the thirty-third day stage the ganglion in the middle third of the heart is still more distinct. The cells are more numerous. The connective tissue covering of the adult ganglion begins to make its appearance. But the ganglion has not yet extended fully to the posterior end and the anterior end. Nor can the lateral nerve cords be made out with a certainty. The growth of the axis

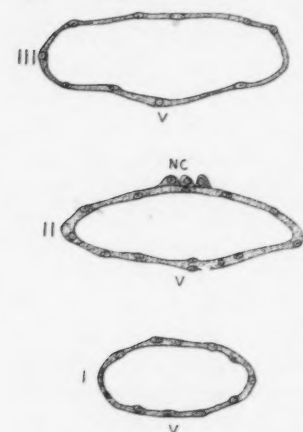


FIGURE 4. — Cross section (semi-diagrammatic) of the embryonic heart of *Limulus* at about the twenty-eighth day stage. I, posterior region; II, middle region; III, anterior region of heart. V, ventral side. NC, nerve-cord anlage in middle region only.

cylinders evidently lags behind the appearance of the cell bodies. At this stage the heart muscle is distinctly cross-striated. But the heart walls are still thin. In fact, they may still be said to be composed of a single layer of elongated and flattened cells. Our observations did not extend beyond the thirty-three day stage.

3. According to all evidence, therefore, there is in the case of *Limulus* a transfer of function between the two heart tissues during embryonic development. There is no question but that the normal rhythm of the adult heart is neurogenic. And as the beginning of the rhythm of the embryonic heart, according to our observations, precedes the development of the heart ganglion, the heart rhythm may be said to be myogenic. At what stage and in what manner is this change of mechanism effected? How is it effected without



introducing a degree of cardiac inco-ordination inimical to the animal? How has such a condition been brought about in phylogeny? Is there any advantage in the neurogenic mechanism over that of the myogenic for the conditions of adult life, or is the change a necessary result of tissue differentiation? Our results suggest more difficult questions than they answer.

In discussion of the nature of the heart rhythm at the meeting of the American Physiological Society at Ann Arbor, in 1905, Dr. J. S. Meltzer suggested that the heart rhythm may be neither wholly neurogenic nor wholly myogenic; it may be both at the same time. It is difficult to conceive of the possibility of such a condition. It is possible, however, that the following conditions obtain. As the muscular automatism



FIGURE 5.—Cross section of middle region of the embryonic heart of *Limulus* at about the thirty-third day, showing distinct transverse striation of the myocardium and a greater development of the nerve cord (NC).

diminishes and the nervous automatism increases in the course of embryonic development, the refractory state of the heart muscle probably prevents any extensive cardiac inco-ordination, as the nervous discharge from the ganglion cells would be practically ineffective until the nervous automatism exceeded in rate and intensity that of the myocardium. When this stage is reached and the heart nervous tissue once gets the lead, the rhythm would continue by the neurogenic mechanism, despite the fact that the myocardium may still be capable of independent automatism should the nervous mechanism in some way break down. There seems to us no reason why these conditions should not persist to some degree even in the adult heart or parts of the heart, so that when the nervous mechanism is interfered with, either experimentally or by pathological processes, the abnormal conditions thus developed cause the original automatism of the myocardium to come to light, even if only in a transitory way. The adult *Limulus* heart has differentiated even beyond this stage, inasmuch as the heart muscle will not initiate a rhythm of its own after removal of the ganglion, no matter how long the metabolites and the carbon dioxide are allowed to accumulate, or how well the muscle is supplied with its normal pabulum. Nevertheless, even the *Limulus* heart muscle exhibits a greater tendency to automatism and rhythmicity

than the adult skeletal muscle. It is possible that the myocardium of some of the vertebrate groups is less differentiated, and therefore retains a greater degree of its original automatism, than does the *Limulus* myocardium. This is indicated by the fact that the *Limulus* myocardium appears to be more distinctly cross-striated than that of the vertebrates.

#### IV. SUMMARY.

1. The beginning of the heart rhythm in *Limulus* embryos occurs on about the twenty-second day after the eggs are laid. At this stage the heart tube is composed of a single thin-layered syncytium in which no fibrillæ or cross-striations can be made out. There is no evidence of the anlage of the dorsal nerve cord, the dorsal nerve plexus, or the lateral nerves in the heart at this stage.

2. The first appearance of the nerve cord in the middle third of the heart can be made out at about the twenty-eighth day stage. But even at this stage the lateral nerves are not in evidence. The myocardium is faintly cross-striated. At the thirty-third day stage the transverse striation of the heart muscle is very distinct.

3. As the conduction and automatism in the adult *Limulus* heart are neurogenic, there must take place a transfer of automatism and conductivity from the myocardium to the nervous tissue at some stage in the embryonic development.



# THE CONDUCTIVITY PRODUCED IN THE NON-CONDUCTING MYOCARDIUM OF LIMULUS BY SODIUM CHLORIDE IN ISOTONIC SOLUTION.

BY A. J. CARLSON.

[From the Marine Biological Laboratory, and the Hull Physiological Laboratory of the University of Chicago.]

## I.

IT has been shown, in previous papers in this journal, that in the Limulus heart the contraction is not conducted through the myocardium. The conduction takes place through the ganglion and the nerve plexus surrounding the myocardium. That this is a fact is a matter, not of inference, but of direct demonstration. The myocardium may be transected, leaving the nerve plexus nearly intact, and conduction is not interfered with. On the other hand, if we transect the nerve net, leaving the myocardium intact, the contraction does not pass the region of the lesion.

Many of the experiments on the mechanism of conduction were made on hearts from which the automatic ganglion had been removed. The normal tonus of the Limulus myocardium is dependent on the automatic ganglion. This fact suggests the possibility that when the heart ganglion is extirpated the myocardium may cease to conduct because of loss of tone. But the evidence is conclusive that the myocardium neither conducts nor is capable of conduction in the intact and normal heart. For example, if mechanical or electrical stimulation is applied to the heart wall in such a way that the ganglion is not involved, the contraction is always confined to the area stimulated. If the stimulation touches the ganglion, the contraction spreads over the whole heart. This shows conclusively that even in the intact and normal heart the contraction cannot spread by muscular conduction beyond the region directly stimulated.

The singular fact was observed, however, that when the ganglion-free Limulus heart is bathed in an isotonic sodium chloride solution

the sodium chloride contraction spreads by conduction through some mechanism different from the normal. The sodium chloride produces no stimulation of the nerve plexus and lateral nerves when isolated from the ganglion, nor is it stimulated locally by the muscle contracting in sodium chloride, and the contraction thus conducted to other parts of the heart. It was suggested that "the sodium chloride alters the muscular tissue so that a wave of contraction is conducted from muscle cell to muscle cell, a process which does not take place normally; or the conduction is effected by means of an intercellular nerve net so related to the plexus connecting the ganglion with the heart wall that the impulses do not pass from the former to the latter."<sup>1</sup>

These observations have now been extended, with the result that the first alternative turned out to be the correct one. Sodium chloride produces conduction in the normally non-conducting myocardium just as it produces a transient automaticity in the non-automatic heart muscle.

## II.

Why is the normal myocardium of *Limulus* incapable of physiological conduction? Does the cause lie in the presence of non-conducting sarcolemmæ, or some non-conducting substance separating the muscle cells from one another? So far as I know, the *Limulus* heart muscle has not been studied as regards these points. All the recent work on the structure of the vertebrate heart muscle seems to show that the myocardium is to be regarded as a syncytium, the muscle fibrillæ passing uninterrupted from cell to cell, or, rather, from one region to another.<sup>2</sup> In this manner the entire myocardium appears to be in protoplasmic continuity. This fact has been construed as a strong argument in favor of the theory of myogenic conduction of the contraction in the normal heart. It has been argued that in such a protoplasmic continuity we have the same anatomical basis for conduction as in the case of conduction of the contraction from one end to the other in a single cell of skeletal muscle. In skeletal muscle, so far as we know, the fibrillæ extend from one end of the cell to the other. There is no evidence of lateral anastomosis. We have, moreover,

<sup>1</sup> CARLSON: This journal, 1907, xvii, p. 486.

<sup>2</sup> The literature is reviewed in detail by RENANT and MOLLARD: *Revue générale histologie*, 1905, i, p. 143.

no evidence that there is an actual continuity of any single fibril extending over the whole heart or even over any considerable portion of the heart. Again, the sarcoplasm may be necessary for physiological conduction in the muscle, and there is evidence that the sarcoplasm suffers some interruption at the original boundaries of the heart cells. The existence of the muscular syncytium in the vertebrate heart is therefore not a demonstration of the myogenic theory of conduction, but it must be admitted that it seems to lend some support to that view.

At my suggestion Mr. W. J. Meek began the study of the structure of the *Limulus* heart muscle at the Marine Laboratory the past summer. His results will soon be published. I have examined many of his preparations. These show conclusively that the myocardium of *Limulus* is a syncytium practically indistinguishable from that of the vertebrate heart. To be sure, the *Limulus* heart muscle is more distinctly cross-striated. The *Limulus* heart muscle cells branch, and the fibrillæ pass without interruption from one muscle cell to the other, or, more correctly, from one strand of the syncytium to the neighboring ones. Mr. Meek has so far not been able to make out any tissue comparable to the Purkinje cells of the vertebrate heart.

The *Limulus* myocardium presents therefore the same anatomical basis for physiological conduction as does the vertebrate heart. The uninterrupted passage of the muscle fibrillæ from neighboring strands of the syncytium gives, apparently, a protoplasmic continuity to the whole myocardium. Nevertheless we have seen that the *Limulus* myocardium does not conduct under normal conditions.

The presence of a muscular syncytium in the *Limulus* heart, coupled with the absence of muscular conduction, renders untenable the argument from the muscular syncytium in support of the myogenic theory of conduction in the vertebrate heart. It also renders pertinent the question of the relation of the sarcoplasm to physiological conduction in muscle cells.

### III.

Histological examination of the nervous tissues in the *Limulus* heart gives no indication of the presence of a nerve net or plexus peripheral to the great plexus that connects the ganglion with the heart muscle. Unless such second plexus is present in the myocar-

dium, it is obvious the conduction of the contraction in the ganglion-free heart beating in sodium chloride is myogenic.

For this work I used mostly very young *Limuli*, because when these small hearts are slit open in the median line on the ventral side they may be spread out in their entirety on the slide and mounted as surface preparation in glycerine or balsam, thus rendering examination of every part of the heart a relatively easy task. These surface preparations of very small hearts are thin enough to be studied by aid of the one-seventh objective, and in some cases even the one-

twelfth oil immersion. Examinations were also made of cross sections of the heart after methylene blue staining of the fresh tissue and fixation in ammonium molybdate

Methylene blue in sea water brings out the thicker branches of the nerve plexus very satisfactorily, but the terminal ramifications of the fibres and their endings on the myocardium are not stained with the same distinctness and constancy. But a sufficient number of successful preparations were obtained to render it certain that the fibres in the great plexus pass directly to the myocardium without making connection with any nerve net situated in closer proximity to the muscle cell.

The fibres in the nerve net are all non-medullated. The different fibres vary considerably in thickness. In surface preparations of very small hearts the thick fibres can sometimes be followed from their origin — a unipolar or multipolar cell in the ganglion — throughout

their whole course to the end on the muscle. The thick fibres bifurcate almost at every point of anastomosis or division of the nerves. In no case have I observed the two branches passing out the same nerve twig. At the place of bifurcation of the fibres is usually found a great thickness of the axis cylinder (Fig. 1 A). These thickenings appear to be identical with those described in vertebrate heart plexus. In *Limulus* these protoplasmic masses frequently attain the size and form of some of the smaller cells in the ganglion, and may easily be mistaken for ganglion cells. The smaller fibres also bifurcate. As the fibres spread over and among

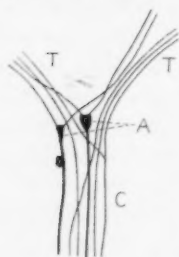


FIGURE 1. — Camera lucida drawing of the course of the fibres at a point of anastomosis of the branches of the nerve plexus in the *Limulus* heart. A, protoplasmic enlargement at the point of bifurcation of the fibre. C, nerve branch coming from the ganglion. T, branches going to the myocardium.

the strands of muscle, they may be frequently seen to branch again, but I was unable to discover anastomosis of these terminal branches with those from neighboring fibres. The fibres appear to end freely on the muscle strands.

We have therefore no anatomical basis for the hypothesis that the conduction of the sodium chloride rhythm in the heart takes place through a nerve net situated in closer proximity to the heart muscle than the plexus superficial to the myocardium. This superficial nerve plexus forms a network over the entire heart tube, but the anastomosis is apparent only, as the junction of the fibres are points of division of the axis cylinders. As the fibres leave this plexus to ramify over the muscle, they are terminal fibres and do not form a second plexus about the muscle strands. The superficial nerve net including the ganglion in the *Limulus* heart is therefore homologous to the intermuscular nerve net in the vertebrate heart.

#### IV.

The evidence that when separated from the ganglion the cardiac nerve plexus takes no part in the sodium chloride rhythm may be summarized as follows:

1. When part of the plexus is isolated and immersed in isotonic sodium chloride, no contractions appear in the part of the heart muscle left in physiological connection with the plexus. I have made numerous experiments to test this point, because in this regard the motor nerve plexus of the *Limulus* heart differs from the peripheral motor nerves of the frog. I was reluctant to have to report such a difference. If sodium chloride stimulates the frog's sciatic in such a way that the gastrocnemius is thrown into an irregular rhythmic activity or incomplete tetanus, there seems to be no good reason why sodium chloride should not do the same in the case of the motor heart nerves in *Limulus*. The fact is, however, that it does not.

There are conflicting statements in the literature regarding the action of sodium chloride on the frog's motor nerves. Loeb states that the frog's sciatic is not stimulated, while Mathews<sup>3</sup> found that the chloride stimulates after a latent period of two hours or more. Maxwell<sup>4</sup> reports the same negative results as Loeb. I have tested the question on five sciatic-gastrocnemius preparations, and obtained

<sup>3</sup> MATHEWS: This journal, 1904, xi, p. 455.

<sup>4</sup> LOEB: *Archiv für die gesammte Physiologie*, 1907, cxvi, p. 194.

positive results in each case. After a latent period of an hour and a half to two hours the muscle begins to twitch more or less rhythmically, and the contractions cease as soon as the portion of the nerve in the solution is cut away (Fig. 2).

## V.

The heart with the ganglion extirpated may be tied off by a coarse thread in the region of the third and sixth segments, thus dividing the heart tube into three separate chambers. If the threads are drawn just tight enough to close the lumen of the heart and prevent

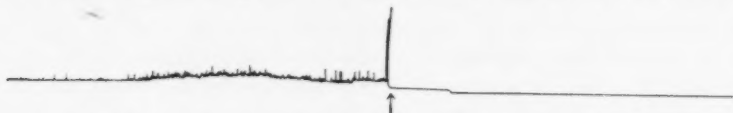


FIGURE 2. — Tracing of the contractions of the frog's gastrocnemius after two hours' immersion of the sciatic nerve in isotonic sodium chloride. ↑, section of the nerve between the sodium chloride bath and the muscle. Showing stimulation of the frog's sciatic by sodium chloride in isotonic solution.

liquid from passing from one of these artificially produced chambers to the other, the compression is not sufficient to interfere with conduction in the nerve plexus, which is included with the myocardium in the ligature. That the compression does not block conduction in the plexus can be readily demonstrated by electrical stimulation of the latent nerves in the fourth segment, as this causes contraction of the anterior and posterior ends of the heart. When isotonic sodium chloride is introduced into the middle compartment of this heart, a violent rhythm is set up in this region within a few minutes, in some cases almost at once. But this rhythm is confined to the region between the ligatures. The two end regions remain quiescent, despite the fact that the conductivity of the nerve plexus is not interfered with.

## VI.

Isotonic sodium chloride alters the property of the *Limulus* myocardium in such way that it not only becomes automatic, but so that the contractions spread from one point over the whole heart by conduction through the muscle substance.

The fact that the sodium chloride alters conduction in the myocar-

dium can be demonstrated even before the sodium chloride rhythm appears. If the collapsed heart is placed in the solution, there is a long latent period (thirty-five to forty-five minutes) before the rhythm begins. It has been shown in a previous report that during this latent period the excitability of the heart muscle is gradually increased up to the point of initiation of the rhythm. This is shown by stimulation of the nerve plexus. On direct stimulation of the heart walls during the latent period the contractions called forth by the same strength of stimulus not only become stronger, but they gradually extend for a greater distance from the point stimulated. In other words, the physiological conductivity of the myocardium is increased.

While a segment or portion of the ganglion-free *Limulus* myocardium immersed in sodium chloride usually exhibits a very irregular rhythm, preparations were occasionally obtained in which the transient sodium chloride rhythm exhibited as perfect a regularity as the normal rhythm. Such regular rhythm involves a co-ordination between the different parts of the segment or preparation, and as co-ordination does not take place through the nerve plexus, it must be effected by conduction in the myocardium.

When the isotonic sodium chloride is poured into the cavity of the heart instead of immersing the collapsed heart in the solution, the rhythm begins after a very brief latent period. The rhythm thus developed never attains the regularity of the normal heart rhythm. In many cases the musculature around the ostia beats faster than the rest. But there may frequently be seen contraction waves passing over the heart, or greater part of the heart, in the form of peristaltic waves. These contraction waves may pass either postero-anteriorly or antero-posteriorly. Occasionally contraction waves may start practically at the same time in the opposite ends of the heart, both of them travelling towards the middle. The rate and direction of these peristaltic waves of contraction may remain constant for considerable periods, but sooner or later irregularities set in in the way of contraction waves coming in the opposite direction, partial contraction, etc.

Such peristaltic irradiation of the contractions never takes place in the normal heart from which the ganglion has been removed. Its presence in the sodium chloride automatism is therefore due to the conductivity of the myocardium induced by the sodium chloride.

The rate of propagation of the sodium chloride peristalsis is



variable, but never exceeded 1 to 2 cm. per second. This is very much slower than the normal rate of conduction in the nerve plexus, although the latter rate is only about 40 cm. per second.<sup>5</sup>

I do not desire at this time to discuss the possible or probable mechanism by which the sodium chloride effects this change of physiological conduction in the myocardium. It is possible that the conduction is simply the result of the increased excitability of the muscle, so that the action current of one muscle strand or fibrilla is able to stimulate its neighbor. This would be analogous to the stimulation of one axis cylinder by the action current of neighboring fibres in a nerve trunk. It is well known that such an irradiation takes place only when the excitability of the nerve fibres has been increased above the normal.

<sup>5</sup> CARLSON: This journal, 1906, xv, p. 101.



## A NOTE ON THE REFRACTORY STATE OF THE NON-AUTOMATIC HEART MUSCLE OF LIMULUS.

By A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

IN my recent paper on the mechanism of the refractory period of the heart<sup>1</sup> it was shown that there is no evidence of causal relation between the property of refractory state and the property of automatism. It was furthermore pointed out that "the question whether heart muscle when isolated from the intrinsic nervous tissues exhibits the property of refractory state to a greater degree than skeletal and smooth muscle is still an open one, since the facts bearing on the question can be interpreted in either way." In the vertebrates the myocardium cannot be separated from the nervous tissue, so that all the evidence bearing on the point for the vertebrate heart is indirect. In *Limulus* the heart ganglion exhibits the typical systolic refractory state in the sense of diminished excitability. The same is true of the myocardium and the nerve plexus as long as they are in physiological connection with the ganglion.

1. The fact that the nerve net takes no part in the sodium chloride rhythm of the heart from which the ganglion has been extirpated<sup>2</sup> offers an opportunity to determine whether or not the non-automatic *Limulus* heart muscle exhibits any marked degree of refractory state. Experiments on this point were carried out at the Marine Biological Laboratory in July, with positive results. The *Limulus* myocardium in sodium chloride rhythm exhibits the typical systolic refractory state in the sense of diminished excitability. This refractory state is in evidence during the systole of the beat produced by direct stimulation with the single induction shock even before the sodium chloride rhythm appears.

2. The various forms of the sodium chloride rhythm of the *Limulus* heart muscle have been described elsewhere. After having

<sup>1</sup> CARLSON: This journal, 1907, xviii, p. 71.

<sup>2</sup> CARLSON: This journal, 1907, xvii, p. 478.

found that the heart muscle exhibits systolic refractory state typical for the heart, I made it a point to determine whether this refractory state is greater in the heart muscle than in the *Limulus* skeletal muscle. Isotonic sodium chloride produces more or less rhythmical twittings in the *Limulus* skeletal muscle, but no rhythm comparable with that induced in the myocardium. Strips of skeletal muscle from different regions of the body were tried. The musculature of the back is the most favorable, as strips can be obtained from

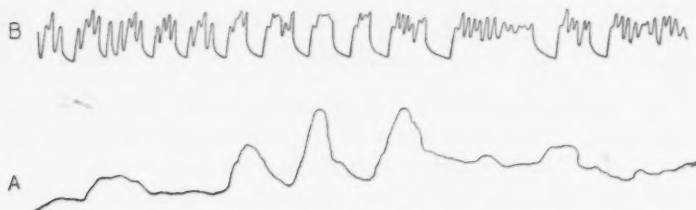


FIGURE 1. — One half the original size. *A*, typical tracing from a strip of *Limulus* skeletal muscle immersed in isotonic sodium chloride. Showing rhythmical twittings on the slower tonus rhythms. *B*, tracing of a very irregular sodium chloride rhythm of the *Limulus* heart muscle. Showing tonus rhythm and fundamental rhythm.

5 to 6 cm. in length and only 3 or 4 mm. in diameter. But the slender strands exhibited just as irregular twittings as the thicker strands. The strips usually exhibited a double rhythm, — if the term rhythm may be applied to the more or less rhythmical twitches, — a distinct tonus rhythm, on which the more rapid twitches were superimposed. These may be called fundamental and tonus rhythms by analogy from the heart. The best tracings of the sodium chloride rhythm of the skeletal muscle exhibit much greater irregularities in strength and rate of contraction than the most irregular rhythm of the heart muscle (Fig. 1). No data could therefore be secured for this comparison.

3. The systolic refractory state can be demonstrated in the *Limulus* heart muscle in sodium chloride rhythm, even though this rhythm is quite irregular, but the most conclusive results are obtained in preparations that exhibit a rhythm approaching in regularity that of the normal. In the case of such preparations a single induction shock of an intensity that produces a shortening of the diastole and a supermaximal beat when sent through the tissue near the end of the diastole has no apparent effect when it reaches the tissue during systole or the first part of the diastole. But this refrac-

tory state is relative only. A greater strength of the induced shock produces supermaximal beats or extra beats, no matter in what phase of the automatic beat it is sent through the preparation. A typical tracing showing the diminished excitability during systole and the greater part of diastole is reproduced in Fig. 2.

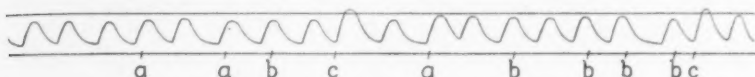


FIGURE 2.—One half the original size. Tracing from a ganglion-free segment of the *Limulus* myocardium in sodium chloride rhythm. Stimulation of the muscle with the make induced shock of uniform intensity: *a*, during systole; *b*, during diastole; *c*, near the end of diastole. Showing a systolic refractory state.

In some of the preparations I found a peculiar modification of the refractory state that must be noted in brief. In the normal rhythm of the *Limulus* or the vertebrate heart the diminution in excitability is greatest at the very beginning of systole, and the excitability returns gradually, till at the end of diastole it has reached its maximum. The *Limulus* myocardium in sodium chloride rhythm not infrequently exhibits a greater excitability at the very beginning of sys-

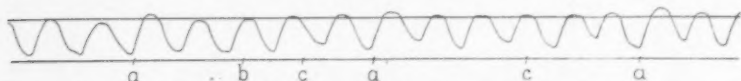


FIGURE 3.—One half the original size. Tracing from a ganglion-free segment of the *Limulus* heart in sodium chloride rhythm. Stimulation with the break induced shock of uniform intensity: *a*, at the very beginning of systole; *b*, later in systole and in diastole. Showing an apparent greater excitability at the very beginning of systole than during the rest of systole and the greater part of diastole. Explanation in text.

tole than during the later stages of systole or the beginning of diastole. A strength of the induced shock may be found that produces a supermaximal beat when sent through the preparation at the beginning of systole, but has no effect when applied later in the systole or in early diastole. A tracing showing this singular distribution of the refractory state in reference to the phases of the beat is reproduced in Fig. 3.

It is probable that in the preparations exhibiting this peculiar condition the muscle does not begin to contract at the same time throughout the whole myocardium. The contraction probably begins at the ostia, and spreads thence over the rest of the myocardium by the slow conduction typical of the conductivity of *Lim-*

ulus heart muscle in sodium chloride.<sup>3</sup> Under such conditions the stimulus reacting on the preparation at the very beginning of systole would strike part of the musculature, not in systole, but at the very end of diastole. This would not be the case with stimuli reaching the preparation during the later stage of systole or the beginning of diastole. This condition of the refractory state never appears in the normal rhythm of the *Limulus* heart, for the reason that the myocardium in any one region of the heart begins to contract practically at the same time throughout its whole extent, owing to the simultaneous distribution of the motor nervous impulses. The condition is practically the same in the vertebrate heart, whether the conduction is myogenic or neurogenic.

We have here for the first time a demonstration of the presence of the refractory state in the heart muscle apart from the intrinsic nervous tissue. Moreover, this is in the case of a myocardium which is non-automatic normally. This lends further support to my contention that there is no causal connection between the property of automatism and the property of refractory state. The refractory state is probably a characteristic of all excitable tissues, some exhibiting it to a greater degree than others. But the property of automatism depends on other — at present unknown — factors.

<sup>3</sup> CARLSON: This journal, 1907, xxi, p. 11.

## THE RATE OF OXIDATION OF SUGARS IN AN ACID MEDIUM.

By H. H. BUNZEL.

*[From the Laboratory of Biochemistry and Pharmacology of the University of Chicago, and the Marine Biological Laboratory, Woods Holl, Mass.]*

### I. INTRODUCTION.

IT has been shown, by Mathews<sup>1</sup> and McGuigan,<sup>2</sup> that acetic acid, when added to a system containing cupric acetate and sugar in solution, reduces the rate of oxidation for two reasons:

1. It probably depresses the dissociation of the sugar molecule into those active particles which are mainly concerned in the oxidation.

2. It yields H ions which reduce the number of the free O ions, and thus diminish the oxidizing power of the cupric acetate solution.

It has also been shown that if sufficient quantities of acetic acid are added to such solutions, perceptible oxidation within a short interval of time may be prevented. The amount of the acid which is just sufficient to prevent the oxidation varies with different sugars, and increases with the ease with which the sugars are being oxidized. According to the quantities of acetic acid required to prevent a perceptible oxidation under the same conditions, the sugars could be arranged in the following order: levulose (most acid required), galactose, glucose, maltose, and lactose (least acid required).

The object of this investigation was to determine more accurately the rate at which the different sugars oxidize in an acetic acid solution, and to establish, if possible, a numerical relationship between the velocities of oxidation of the different sugars. The experiments were made with levulose, glucose, galactose, mannose, maltose, and lactose. For all experiments Kahlbaum's purest preparations of the different sugars were used. Saccharose was not studied, as it is partially inverted in the course of an experiment,

<sup>1</sup> MATHEWS, A. P., and MCGUIGAN: This journal, 1907, xix, p. 199.

<sup>2</sup> MCGUIGAN, H.: This journal, 1907, xix, p. 175.

and this, with its very slow rate of oxidation, makes it impossible to study the oxidation of the saccharose itself. In the case of lactose and maltose, as will be seen later, the inversion was slow enough not to interfere with the measurement of the primary oxidation.

## II. METHODS.

A simple method was devised, which allowed the removal of successive portions of the mixture without disturbing the conditions under which the oxidation took place. The use of the apparatus shown in Fig. 1 is self-evident. *A* is a 250 c.c. flask, to be filled with the sugar-cupric acetate mixture; *B* is a bent tube with stopcock, by means of which some of the liquid may be drawn off at any time during the course of an experiment; and *C* is a dropping-funnel, kept almost full of mercury, to force the liquid through the tube *B* when the stopcock *E* is opened, and to act as a safety valve; *D* is a thermometer by means of which the temperature within the flask is determined. The apparatus was placed in a wire basket which was suspended in a large basin of boiling water. With such an apparatus it is possible to draw off large or small samples of the liquid at any time during the course of an experiment without the possibility of any change in the concentration of any one of the constituents of the system by evaporation.

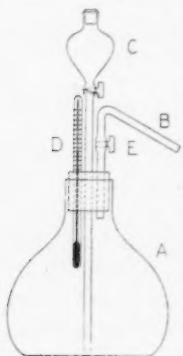


FIGURE 1.

The manipulation was very simple. Successive portions were withdrawn for analysis at definite time intervals. After discarding the first 2-3 c.c., the solution was run directly from the flask into a 25 c.c. burette, in which it was cooled at once under the tap. This practically stopped the oxidation. The copper in the liquid was now determined by titration with one-tenth normal thiosulphate solution, using starch emulsion and potassium iodide as an indicator. The end point was said to be reached when the mixture assumed a pure white color and turned blue on addition of one drop of the cupric acetate solution. To prepare the oxidizing solution, distilled water was saturated with cupric acetate, and the copper determined in the solution, after having filtered the latter. This was diluted with water and 8 N acetic acid until the concentration was  $m/2$  with respect to the acetate and normal with respect to

the acid. The sodium thiosulphate solution was standardized by means of a one-tenth normal iodine solution.

To make certain that the mercury took no part in the reaction, some control experiments were made by means of the sealed-tube method used by Mathews and McGuigan. Glass tubes of about 1 cm. diameter and 20 cm. long, closed at one end, were almost

TABLE I.  
Concentration of  $\text{Cu. } \frac{m}{4}$ , sugar  $\frac{m}{20}$ , acetic acid  $\frac{m}{2}$ .

Time elapsed between be- ginning of ex- periment and withdrawal of liquid.	Concentration of Cu in terms of $\frac{n}{10} \text{Na}_2\text{S}_2\text{O}_3$ solution.		
	Using sealed tubes.	Using improved method.	Difference.
min. 0	2.520	2.500	0.020
10	1.530	1.522	0.008
20	1.103	1.086	0.017
30	0.854	0.805	0.049
40	0.630	0.639	0.009
50	0.525	0.519	0.006
60	0.439	0.442	0.003

completely filled with the solutions, and then the other end sealed. They were all put into a bath of boiling water at the same time, and removed one by one at the desired time intervals. The tip of the tube was then broken off and the concentration of the unreduced copper determined. The results are given in Table I.

The deviations seen in Table I. are within the range of experimental errors, and show that the mercury played no part in the reaction.

### III. MEASUREMENTS OF THE RELATIVE SPEEDS OF OXIDATION OF THE DIFFERENT SUGARS.

The method being correct, I proceeded to measure the rate at which the different sugars reduce the cupric acetate-acetic mixture. The results are given in Table II. In all these experiments the mixture contained at the beginning of the oxidation the sugar at a concentration of  $m/20$ , copper acetate  $m/4$ , and the acetic acid  $m/2$ .



TABLE II.

Sugar.	Time.	Number of c.c. $\text{Na}_2\text{S}_2\text{O}_3$ sol. required to titrate 1 c.c. of the Cu solution.	Time elapsed since preceding determination, $t$ .	Amount of Cu reduced in time $t$ expressed in c.c. of a $\frac{N}{18}$ $\text{Na}_2\text{S}_2\text{O}_3$ sol.	Mean velocity during $t$ . Amount reduced $\div t$ .
Levulose	4 : 25	placed in bath	of boiling water.		
	4 : 31	2.500	0'	....	....
	4 : 40	1.522	9'	0.978	0.1087
	4 : 50	1.086	10'	0.436	0.0436
	5 : 00	0.805	10'	0.281	0.0281
	5 : 10	0.637	10'	0.168	0.0168
	5 : 20	0.519	10'	0.118	0.0118
	5 : 30	0.442	10'	0.077	0.0077
	5 : 40	0.381	10'	0.061	0.0061
	5 : 50	0.337	10'	0.044	0.0044
	6 : 00	0.293	10'	0.040	0.0040
Galactose	2 : 41	placed in bath	of boiling water.		
	2 : 48	2.500	0'	....	....
	2 : 50	2.425	2'	0.075	0.0375
	3 : 00	2.144	10'	0.281	0.0281
	3 : 15	1.809	15'	0.335	0.0223
	3 : 35	1.568	20'	0.241	0.0120
	3 : 55	1.402	20'	0.166	0.0083
	4 : 30	1.213	35'	0.189	0.0054
	5 : 00	1.086	30'	0.127	0.0042
	5 : 30	0.984	30'	0.102	0.0034
	6 : 00	0.894	30'	0.090	0.0030
	7 : 00	0.769	60'	0.125	0.0021
	8 : 00	0.646	60'	0.123	0.0020
Glucose	11 : 10	placed in bath	of boiling water.		
	11 : 16	2.487	0'	....	....
	11 : 30	2.110	14'	0.337	0.2690
	11 : 45	1.820	15'	0.290	0.0193



*Rate of Oxidation of Sugars in an Acid Medium.* 27

TABLE II (continued).

Sugar.	Time.	Number of c.c. $\text{Na}_2\text{S}_2\text{O}_3$ sol. required to titrate 1 c.c. of the $\text{Cu}_2$ solution.	Time elapsed since preceding determination. $t$ .	Amount of Cu reduced in time $t$ ex- pressed in c.c. of a $\frac{1}{10}$ $\text{Na}_2\text{S}_2\text{O}_3$ sol.	Mean velocity during $t$ . Amount re- duced $\div t$ .
Glucose	12 : 00	1.611	15'	0.209	0.0139
	12 : 20	1.418	20'	0.193	0.0086
	12 : 50	1.208	30'	0.210	0.0070
	1 : 35	1.005	45'	0.203	0.0045
	2 : 30	0.852	55'	0.155	0.0028
	3 : 00	0.790	30'	0.062	0.0021
	4 : 00	0.668	60'	0.122	0.0020
	4 : 15	0.654	75'	0.136	0.0018
Lactose	1 : 40	placed in bath	of boiling water.		
	1 : 48	2.459	0'	....	....
	3 : 15	2.044	27'	0.415	0.01537
	5 : 00	1.641	105'	0.403	0.00384
	6 : 00	1.435	60'	0.206	0.00343
	7 : 45	1.097	105'	0.338	0.00322
	10 : 05	0.802	140'	0.295	0.00211
	10 : 50	0.707	45'	0.095	0.00211
Mannose	2 : 11	placed in bath	of boiling water.		
	2 : 20	2.432	0'	....	....
	2 : 30	2.135	10'	0.297	0.0297
	2 : 40	1.903	10'	0.232	0.0232
	2 : 50	1.756	10'	0.147	0.0147
	3 : 00	1.563	10'	0.193	0.0193
	3 : 10	1.443	10'	0.120	0.0120
	3 : 30	1.280	20'	0.163	0.0081
	4 : 00	1.078	30'	0.202	0.0067
	4 : 30	0.929	30'	0.149	0.0050
	5 : 00	0.820	30'	0.109	0.0036
	6 : 00	0.642	60'	0.178	0.0029
	7 : 00	0.564	60'	0.078	0.0013

If we plot these results, representing times on the ordinates and concentrations on the abscissæ, we get the curves shown in Fig. 2.

An inspection of these curves shows in a striking manner the different velocities with which the various sugars are oxidized. Levulose is oxidized with far greater speed than any of the others. Mannose comes next after levulose. Galactose at the outset is

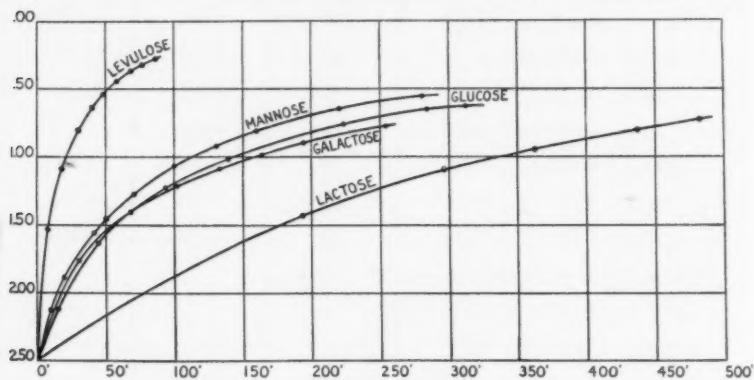


FIGURE 2.

oxidized a little more rapidly than glucose, but later falls behind the latter. Lactose is oxidized much more slowly. These results confirm Mathews and McGuigan both in the relative order the sugars take in their ease of oxidation and also that there are wide differences in the velocities with which the sugars are oxidized. In addition the position of mannose is shown to be intermediate between levulose and galactose.

#### IV. CAN A VELOCITY CONSTANT BE FOUND FOR THESE REACTIONS?

From the decisive character of the preceding results it was first hoped that an equation might be found which would yield a velocity constant by which the oxidation velocities of the different sugars could be numerically expressed. From *a priori* reasoning it did not seem very likely that such a constant could be found, since by the oxidation of a part of the sugar acid is produced, and this, as is shown further on, greatly retards the rate of oxidation. However, as it was possible that this factor might not too greatly in-

terfere, various attempts were made to find equations giving a constant. The attempts, however, were fruitless. The general velocity equation,

$$\frac{dx}{dt} = k(a-x)(b-x)$$

failed, as did also the equations of reactions of the first, third, and fifth order. To determine the order of the reaction, therefore, it was necessary to use a method which would give accurate values for the order of the reaction in the presence of disturbing conditions. Such a method is that of van't Hoff.

#### V. THE DETERMINATION OF THE ORDER OF THE REACTION

To determine  $n$  van't Hoff's differential method was used. This method is based on the fact that the velocity of a reaction of the  $n$ th order is proportional to the  $n$ th potential of the concentration. The conditions are simplest when the substances are present in equimolecular proportions. Then

$$-\frac{dc}{dt} = kC^n,$$

where  $C$  denotes the concentration of the reacting substances.

If we make two experiments with different initial concentrations,  $C'$  and  $C''$ , of the reacting substances, we get

$$(1) \quad -\frac{dc'}{dt} = kC'^n; \quad (2) \quad -\frac{dc''}{dt} = kC''^n.$$

If we divide expression 1 by expression 2, we get

$$\frac{\frac{dc'}{dt}}{\frac{dc''}{dt}} = \left(\frac{C'}{C''}\right)^n.$$

Taking the logarithms of both sides of the equation, we get

$$\log \left(\frac{dc'}{dt}\right) - \log \left(\frac{dc''}{dt}\right) = n \log \left(\frac{C'}{C''}\right).$$

Solving for  $n$ ,

$$n = \frac{\log \frac{dc'}{dt} - \log \frac{dc''}{dt}}{\log C' - \log C''}.$$

For the sake of determining  $n$  experimentally, two solutions were made up of the following compositions:

**Solution I. —**

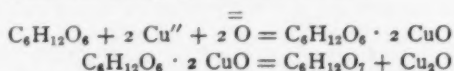
Number of c.c. of $m/4$ copper acetate solution in $n$ acetic acid used . . . . .	100.00
Grams of glucose dissolved to make 100 c.c. of solution . .	4.95
Total volume of the solution expressed in c.c. . . . .	200.00
Molecular concentration of Cu . . . . .	0.1375
Molecular concentration of sugar . . . . .	0.1375
Molecular concentration of acetic acid . . . . .	0.5

**Solution II. —**

100 c.c. of solution A,  
100 c.c. of  $m/2$  acetic acid.

The experiments were conducted in the usual way. The titer was determined at the outset, twenty minutes after placing the reaction flask in the bath of boiling water, and twice more at intervals of ten minutes each. The results are shown in Table III. The figures given for the varying concentrations of the solution represent the number of centimetres of tenth normal thiosulphate solution necessary to titrate 1 c.c. of the cupric acetate solution. The figures in column represent the differences between the initial titer and the titer at the withdrawal of the sample, and stand therefore for the extents of oxidation in the different cases. The figures in column *C* are the mean concentrations for twenty, thirty, and forty minutes respectively.

The figures seem to show that the oxidation of glucose by copper acetate in an acetic acid solution is a reaction of the fifth order. In view of this fact it was suggested to me by Professor Mathews that the reaction might be written in two stages as follows, the first of these equations being of the fifth order: <sup>3</sup>



<sup>3</sup> This equation does not apply strictly to the results obtained, since it involves oxygen.

TABLE III.

Time.	Solution.	No. of c.c. of <i>n</i> 10 thiosulphate solution requ. to titrate 1 c.c. of Cu-sugar mixture			$\frac{dx}{dt}$	C.
		A.	B.	Mean.		
At start.	I.	1.379	1.375	1.377	....	....
	II.	0.686	0.684	0.685	....	....
Twenty minutes after flask has been put into bath.	I.	0.945	....	0.945	0.432	1.1610
	II.	0.664	0.664	0.664	0.021	0.6745
Thirty minutes after flask has been put into bath.	I.	0.832	0.834	0.833	0.544	1.1050
	II.	0.653	0.647	0.650	0.035	0.6675
Forty minutes after flask has been put into bath.	I.	0.742	0.742	0.742	0.635	1.0595
	II.	0.628	0.627	0.627	0.052	0.6560

From these figures

$$n_{20} = \frac{\log .432 - \log .021}{\log 1.1610 - \log .6745} = \frac{1.31326}{.23585} = 5.56$$

$$n_{30} = \frac{\log .544 - \log .035}{\log 1.1050 - \log .6675} = \frac{1.19153}{.21891} = 5.44$$

$$n_{40} = \frac{\log .635 - \log .058}{\log 1.0595 - \log .6560} = \frac{1.03934}{.20800} = 5.00$$

mean value for *n* = 5.33

#### VI. EFFECT OF ACIDITY ON THE RATE OF OXIDATION.

It was found, by Mathews and McGuigan, that the rate of oxidation is greatly slowed by the addition of acetic acid. Further observations on this point were, however, desirable, and the following experiments were tried.

Table IV. gives the concentrations of the solutions used, as well as the results obtained. The mixtures were put into glass tubes, which were then sealed and dropped into a bath of boiling water. After heating the contents for twenty minutes, they were removed, cooled under the tap, and the copper in the solution determined.

In the last experiment some of the acetate was precipitated by mass action.

From Fig. 3, where these results are represented graphically, it is evident that the acidity of the system is a very important factor in the rate with which the oxidation goes on, and that the rate is greatly slowed by the addition of acetic acid. It is seen, as might be expected, that the addition of small amounts of acetic acid slows the reaction greatly, but that increasing the acidity from *N* on produces very little change in the rate. This result evidently means that the addition of more concentrated acid does not increase the number of H ions by any means proportional to the total increase in concentration of acid added.

The reason why the acid reduces the rate of oxidation is probably that already stated at the beginning of the paper, and shown in the hypothetical equation on page 30, *i. e.*, by reducing the number of oxygen ions and dissociated sugar particles.

#### VII. COMPARISON OF THE RATE OF INITIAL VELOCITIES.

While it was found impossible to get any constants which would permit of a numerical representation of the different velocities at which the sugars oxidize, it occurred to us that a comparison of the velocities for a certain short interval of time at the beginning of the reaction would give us an approximation of what we wished. For it is clear that the velocity is probably at its maximum at the start of the reaction, and thereafter steadily diminishes owing to secondary reactions, increased acidity, and the using up of material. The shorter the time interval during which the change in oxidation is taking place and the nearer it is to the beginning of the reaction, the more closely will the result approximate the actual initial velocity of the reaction. Unfortunately, however, it is impossible to measure accurately the change in these short intervals. It is possible, however, to compute approximately the velocities with which the reactions start out. If we prepare a series of solutions, all of

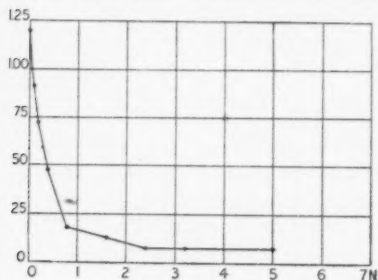


FIGURE 3.

TABLE IV.

Solutions.	C.c. of neutral $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$ solution ( $m/2$ ).	C.c. of 1 per cent glucose solution.	$\text{HC}_2\text{H}_3\text{O}_2$ $\frac{2}{2} n$ .	$\text{H}_2\text{O}$ .	$\text{CuC}_2\text{H}_3\text{O}_2$ .	Titer of solu- tion at begin- ning of the experiment.	Titer after oxi- dation has gone on for twenty minutes.	Amount of reduction.
A	20.00	10.00	....	20.00	....	2.00	0.794	1.206
B	20.00	10.00	2.50	17.50	0.1 $n$	2.00	1.085	0.915
C	20.00	10.00	5.00	15.00	0.2 $n$	2.00	1.282	0.718
D	20.00	10.00	7.50	12.50	0.3 $n$	2.00	1.406	0.594
E	20.00	10.00	10.00	10.00	0.4 $n$	2.00	1.519	0.481
F	20.00	10.00	10.00 $\text{HC}_2\text{H}_3\text{O}_2$ 5.00 8 $n$	15.00	0.8 $n$	2.00	1.826	0.174
G	20.00	10.00	10.00	10.00	1.6 $n$	2.00	1.875	0.125
H	20.00	10.00	15.00	5.00	2.4 $n$	2.00	1.917	0.083
I	20.00	10.00	20.00 Glacial	....	3.2 $n$	2.00	1.913	0.087
J	20.00	10.00	15.00	5.00	5.0 $n$	2.00	1.928	0.072
K	20.00	10.00	20.00	....	6.66 $n$	2.00	1.849	0.151

which contain the same amount of copper and acetic acid, and each one of which contains a different sugar in the same molecular concentration, and allow the oxidations to go on under the same conditions, we can, by interpolation from the curves obtained in

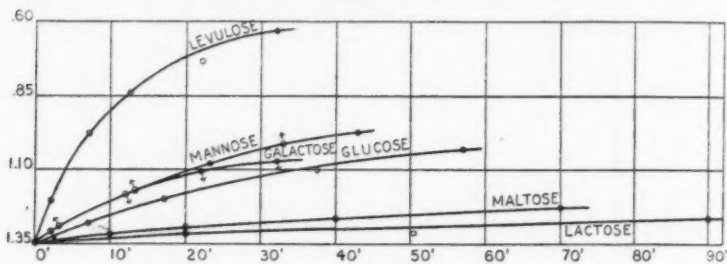


FIGURE 4.

this way, compute the average velocities in the first small element of time. We can thus get definite figures, which would be an approximate measure of the relative rate of the oxidation of the sugars. It would not be an absolute measure of the relative veloc-

TABLE V.

Glucose.		Levulose.		Galactose.		Mannose.		Maltose.		Lactose.	
Time.	Titer.	Time.	Titer.	Time.	Titer.	Time.	Titer.	Time.	Titer.	Time.	Titer.
min.		min.		min.		min.		min.		min.	
0	1.352	2	1.200	2	1.320	3	1.301	0	1.356	0	1.360
7	1.286	7	0.978	12	1.180	13	1.174	10	1.338	20	1.322
17	1.202	12.5	0.848	22	1.111	23	1.088	20	1.315	50	1.316
37	1.098	22	0.729	32	1.070	33	1.019	40	1.284	90	1.264
57	1.026	32	0.624	..	....	43	0.982	70	1.246	..	....

ity of oxidation, on account of the changes in ease of oxidation during the course of a reaction, which changes in the different sugars are by no means parallel.

In Table V the results are given in the usual way. The solutions contained 1 molecule of sugar to 6 of the copper; the acidity was  $m/2$ .

If we calculate the average velocities at five-minute intervals from the curve in Fig. 4 and plot the figures obtained, representing



# *Rate of Oxidation of Sugars in an Acid Medium.* 35

times on the ordinates and the average velocities on the abscissæ, the points of intersection on the y axis will give us our initial velocities. From Figs. 5 and 6 it is evident that these values in the case of the different sugars are in the following ratio:

Lactose . . . .	0.00195	Galactose . . . .	0.01700
Maltose . . . .	0.00225	Mannose . . . .	0.01700
Glucose . . . .	0.01120	Levulose . . . .	0.10750

Assuming that the velocity of the oxidation in the case of lactose is unity, we obtain the following figures:

Lactose . . . .	1.00	Galactose . . . .	8.72
Maltose . . . .	1.15	Mannose . . . .	8.72
Glucose . . . .	5.71	Levulose . . . .	55.13

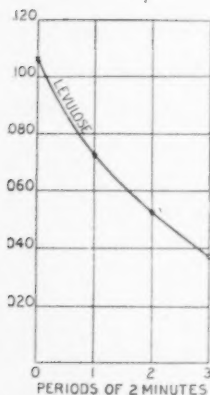


FIGURE 5.

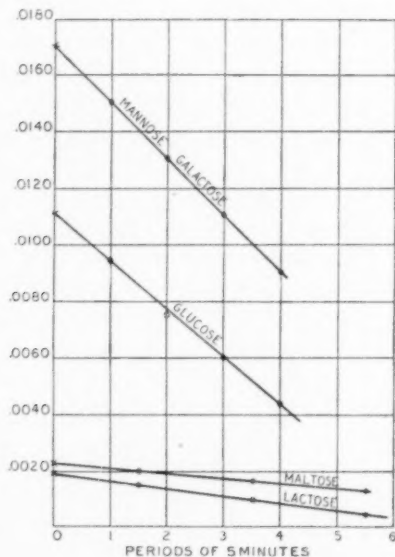


FIGURE 6.

If the velocity is proportional to the amount of dissociation of the sugar molecules as suggested by Mathews and McGuigan, these figures mean that in a solution of this degree of acidity levulose is dissociated 55 times as much as lactose, 48 times as much as maltose, 9.7 times as much as glucose, and about 6.3 times as much as galactose and mannose, when in this concentration. These

figures, while necessarily only approximations, are certainly more accurate than those of Mathews and McGuigan, which were computed from solutions of different acidities and which were given only as rough estimates. There is no doubt, however, that they represent fairly the order of the velocities of oxidation under these conditions.

### VIII. SUMMARY AND DISCUSSION.

The experiments described and the conclusions from them may be summarized as follows:

1. The sugars, glucose, levulose, mannose, galactose, maltose, and lactose, may be oxidized in an acid solution ( $n/2$  acetic acid) with such relative initial velocities as are represented in the following:

Lactose (oxidizes slowest)	1.00	Galactose	8.72
Maltose	1.15	Mannose	8.72
Glucose	5.71	Levulose	55.13

It appears to the writer of this paper that this difference in the kinetic state of the sugars might well be accounted for by Nef's theory, as suggested by Mathews and McGuigan, according to which the reactivity of the organic substances is dependent on their extent of dissociation. If this be true, the figures above represent the relative amount of dissociation of the sugars.

2. The reaction is one of the fifth order.

3. H ions retard the oxidation considerably. It is thought that this is due principally to two reasons:

- a. They decrease the total number of free O ions in the solution.
- b. They diminish the dissociation of the sugar molecule.

Under certain conditions it is possible to make use of the facts brought out in the above to estimate the amount of sugar present in a solution. It must be admitted, of course, that the conditions under which such determinations would be possible are limited. We have to prepare a series of solutions containing known amounts of the sugar in question and determine the rate of oxidation in these solutions. By repeating the experiment under similar conditions with the unknown, we can locate the latter in our arbitrary scale.

The writer wishes to express his gratitude to Professor A. P. Mathews, who suggested this problem to him and under whose direction this work has been done.

PERFUSION EXPERIMENTS ON EXCISED KIDNEYS. —  
IX. THE EFFECTS OF VARIOUS POISONS.<sup>1</sup>

By TORALD SOLLMANN AND R. A. HATCHER.

[From the Pharmacological Laboratories of the Medical Departments of Western Reserve University, Cleveland, Ohio, and of Cornell University, New York, N. Y.]

IN this series of experiments we proposed to study the direct effects of a series of poisons on the kidney, and to distinguish in how far these effects are vascular; or whether they are to be referred to changes in the filtering membrane, or to some action on the secretory epithelium. This could be deduced from simultaneous observation of the vein and ureter flow and oncometer; and by subjecting the kidney to more or less severe injuries, which may be supposed to affect one set of structures more readily than another. To this end the perfusions were undertaken with defibrinated blood, diluted with two to four volumes of 1 per cent sodium chloride solution, at body temperature and at room temperature. Somewhat more unfavorable conditions were secured by perfusion with 1 per cent sodium chloride. These conditions were found to approximate the normal results in most cases. More severe injury was caused by sodium fluoride and other poisons and by 2 per cent sodium chloride. The latter procedure secures the advantage of very free ureter flow, facilitating the observation of the effects on filtration. Finally, all vital reaction could be definitely excluded by perfusing the kidneys several days after they had been excised; but the ureter flow in these kidneys is always very scanty.

These conditions were not all tried with each drug, but only those which appeared promising. The technique of the perfusions was that described in the previous papers of this series. We shall present the results in abstract, arranging the drugs alphabetically. The relative strength of the effects will be indicated by descriptive terms; we purposely refrain from expressing these values as per-

<sup>1</sup> The previous papers of this series have appeared in this journal, xiii, pp. 241-303, and xix, pp. 233-257.

centages, since this gives a wholly misleading impression of mathematical accuracy. As a matter of fact, the quantitative effect can be estimated much more correctly by a careful study of the curves than directly from the numerical data. The conclusions from these results are summarized under each drug and compared with those of other investigators. The effects of drugs on the flow through excised organs have been studied by a number of experimenters, who generally employed defibrinated blood. The data up to 1896 have been taken from the convenient compilation of Paldrock.<sup>2</sup> Of the later work we have only quoted that of Sakusoff,<sup>3</sup> who perfused the kidneys with Locke's solution.

1. **Adrenalin.** — A part of the results with this drug were described by us in previous papers.<sup>4</sup> It was used in the uniform concentration of 1 : 50,000. When added to dilute defibrinated blood (seven perfusions through six kidneys), this produced in practically every experiment a very marked decrease of the vein and ureter flow and oncometer, indicating a strong vasoconstriction. The effect occurred very promptly (generally within five minutes) and was very lasting — the recovery being sometimes very incomplete seventy-five minutes after the perfusing fluid had been changed to unpoisoned blood. The delayed recovery is doubtless due to the very slow displacement of the poison from the constricted vessels. This is in marked contrast with the quick disappearance of the vascular effects of an adrenalin injection in living animals. The latter cannot, therefore, be explained by an extensive destruction of the adrenalin in the kidney, nor by fatigue or failure of the vessels to respond to a continuous adrenalin stimulation.

As recorded in the previous communications, the constrictor action of adrenalin diminishes with every injurious influence and may give place to a moderate dilator reaction. This dilator reaction was observed once with the blood solution, and more frequently when the adrenalin was perfused in 1 per cent NaCl. Of thirteen such perfusions (1 adrenalin to 50,000 of 1 per cent NaCl) on nine kidneys, six showed a typical constrictor action; three were ineffective; in three the oncometer fell, but the vein flow increased; and in one the oncometer and vein flow both increased. In the last

<sup>2</sup> PALDROCK: *Arbeiten des Pharmakologischen Instituts zu Dorpat*, 1896, xiii, p. 87.

<sup>3</sup> SAKUSOFF: quoted from the abstract in *Biochemisches Centralblatt*, 1904, iii, p. 40.

<sup>4</sup> T. SOLLMANN: *This journal*, xiii, p. 246, and R. A. HATCHER, *Ibid.*, xv, p. 144.

case an earlier perfusion gave a typical vasoconstriction which passed off during the adrenalin perfusion. In another case the adrenalin perfusion was continued for one hundred and thirty minutes, the constriction persisting through this entire period, although it was somewhat less toward the end.

On the whole, the constrictor reaction was less constant and seemed perhaps, on the whole, somewhat less powerful when the adrenalin was added to the NaCl than when it was added to blood; this last point is difficult to decide. Otherwise, when the constriction occurred, it differed in no observable manner from that obtained in blood.

Hatcher showed that excised kidneys perfused with normal saline solution cease to respond to adrenalin within three hours; when perfused with Ringer's solution, in three to nine hours; whilst after perfusion with Locke's fluid a good reaction could only be obtained after twenty-four hours.

In 2 per cent NaCl the adrenalin in the above proportion was ineffective, — of eleven perfusions through as many kidneys, seven showed no effect whatever; in the other four there was some decrease of the vein or ureter flow or oncometer. This was slight and never involved all three functions, so that it was either insignificant or accidental.

After previous perfusions with 0.3 per cent sodium fluoride in 1 per cent NaCl for one-half to one hour, some constriction occurred when adrenalin was added to the perfusing blood, but this was always slight (three perfusions).

A marked constrictor action was exhibited by all the other organs examined: four perfusions of legs, with 1 per cent NaCl; one of spleen with blood, and one with 1 per cent NaCl; one of intestine with 1 per cent NaCl and one with blood.

*Conclusions.* — Adrenalin in the proportion of 1:50,000 gives marked vasoconstriction in all the organs examined, *i. e.*, diminution of vein flow and oncometer and of the ureter flow. The effect occurs very promptly, and lasts as long as any adrenalin is present, *i. e.*, over several hours. The constrictor effect lessens, however, with the time elapsing after the excision of the organ, and, in the case of the kidney, may give place to dilation.

The constrictor reaction occurs in blood and 1 per cent NaCl and Locke's fluid, but not in 2 per cent NaCl. Previous perfusion with sodium fluoride lessens the effect, but does not abolish it completely.

2. **Alcohol.** — A 1 : 1000 solution in 2 per cent NaCl was perfused through nine kidneys, always for thirty-five minutes. The vein flow was unaltered in five, doubtful in three, and slightly decreased in one. The ureter flow was unchanged in three, doubtful in four, and slightly decreased in one. The oncometer was unchanged in three, doubtful in two, and slightly decreased in one. The doubtful changes are more often in the direction of decrease.

But one perfusion was tried with 1 : 1000 in 1 per cent NaCl on a fresh kidney (twenty minutes); the effect was quite negative. Even a 1 : 100 in 1 per cent NaCl (twenty minutes) was negative in two kidneys, whilst in the third there was a fair decrease of vein and ureter flow, but not of the oncometer.

*Conclusion.* — Alcohol is without effect when perfused in the proportion of 1 : 1000 to 1 : 100 in 2 per cent and 1 per cent NaCl. Kobert also found a 1 to 2 : 1000 solution ineffective on the calf's foot.

3. **Amyl nitrite.** — This was perfused once through an excised leg and once through a recently excised kidney. On the leg a 1 : 5000 in 1 per cent NaCl was used. The result was a marked decrease of vein flow. On the kidney a 1 : 1500 solution in diluted blood was employed. There was a slight, doubtful decrease of vein flow and increase of oncometer. This kidney responded well to chloral; the leg to this and to digitalis.

Whilst these results are too scanty for definite judgment, they certainly speak strongly against a marked peripheral vasodilator action; on the contrary, they rather tend to show vasoconstriction.

Kobert and Thompson obtained a considerable vasodilator effect on kidneys, but they used concentrations from three to eight times as high as the strongest which was employed by us.

4. **Arsenate of sodium.** — Twelve perfusions were made with 1 : 1000 of 2 per cent NaCl. The vein flow was markedly decreased in nine, unchanged in three. The ureter flow was markedly decreased in seven, doubtful in three, unchanged in two. The oncometer fell in five, was unchanged in one. These effects occurred well after 1 : 1000 NaF (two experiments), but not after 1 : 10,000 picric acid (one experiment). Subsequent perfusion with NaCl gave very imperfect recovery of the vein flow and still less of the ureter flow. Of two perfusions of 1 : 1000 in defibrinated blood, one was negative, the other showed a fall of vein flow and oncometer.

*Conclusion.* — The excised kidney therefore shows no indication of the capillary dilation which is considered the most characteristic effect of arsenic in living animals. On the contrary, the vascular resistance is increased. The relatively smaller and less constant effect on the oncometer indicates that this resistance is introduced more peripherally than with mercury or picric acid.

Kobert found 0.4 : 1000 without effect on the kidney.

**5. Caffein.** — This was used in the form of caffein-sodium-benzoate and of the citrated caffein. Since these compounds contain about 50 per cent of the alkaloid, the real concentration is but one half of that stated. We made five perfusions of 1 part of caffein sodium benzoate in 5000 parts of 1 per cent NaCl; six perfusions with the same concentration in diluted blood; and six perfusions with 1 part citrated caffein in 10,000 parts 2 per cent NaCl. These were absolutely negative with a single exception, in which there was a considerable increase of the vein and ureter flow. This must have been accidental.

Three kidney sections were transferred from 1 per cent NaCl to 1 part of caffein sodium benzoate in 5000 parts of 1 per cent NaCl; two of these lost slightly in weight, the other remained unchanged.

*Conclusions.* — In concentrations up to 1 : 10,000, caffein in the form of caffein sodium benzoate and of citrated caffein was quite ineffective, even when perfused with cold blood.

Sakusoff reports vasodilation for the same concentration. Kobert, however, found only a slight dilation with 1 : 1000, but a fair dilation with 1.2 : 1000.

**6. Cantharidin.** — This was only tried on one kidney, but here under the most favorable conditions, namely, in diluted blood (1 : 5000) and soon after the excision of the kidney. The effect on the vein flow and oncometer was entirely negative, indicating that cantharidin does not act on the excised kidney.

**7. Carbon dioxide.** — Blood saturated with the gas was perfused four times; a saturated solution in 2 per cent NaCl twice. The result was entirely negative.

**8. Carbon monoxide.** — Blood saturated with coal gas was perfused through three kidneys. In another experiment the blood was saturated with pure carbon monoxide. The results were uniformly negative.

**9. Chloral hydrate.** — A 1 : 1000 solution in diluted blood was perfused twice through the same kidney, producing in each case a



very marked increase of vein flow and oncometer. The effect began at once, but continued to increase during twenty minutes. On resuming the unpoisoned blood, the effect gradually disappeared. Identical effects were obtained on a leg with 1:500 solution in 1 per cent NaCl; and on a spleen perfused with 1 per cent NaCl, 500 of 10 per cent chloral being injected into the perfusing cannula.

The chloral was found to remove at once the vasoconstriction of barium chloride and digitalis, whilst the latter in its turn promptly abolishes the action of chloral.

*Conclusions.* — Chloral hydrate produces a powerful vasodilation in a concentration of 1:1000, in all the organs tried, and is mutually antagonistic with barium and digitalis.

Sakusoff records dilation with 1:10,000. Morse observes but a slight dilation with 1 to 5:1000. Kobert obtained considerable dilation with concentrations of 2:1000 and upward, in all the organs tried. Paldrock obtained a fair dilation with 0.2:1000.

**10. Digitalin and digitalis.** — A 15 per cent tincture of digitalis added to blood in the proportion of 1 per cent (equals 0.15 per cent of digitalis), caused a prompt and very marked decrease of the vein flow and oncometer in a kidney which had been excised six hours. The action was removed at once by chloral. A 2 per cent dilution of the tincture in 1 per cent NaCl (equals 0.3 per cent of digitalis) very greatly diminished the flow through an amputated leg, four hours after death.

Two samples of digitalin, diluted with two per cent NaCl were tried. One of the samples was perfused through six kidneys for forty minutes in the concentration of 1:5000. The results were entirely negative. The same sample was also perfused for forty minutes through six kidneys in the concentration of 1:1000. In all of these perfusions the ureter flow and oncometer remained unchanged, but the vein flow showed a slight and doubtful decrease in four.

The other sample was perfused through one kidney in the concentration of 1:50,000 and through five as 1:10,000, in 2 per cent NaCl. Practically all of these showed a moderate but distinct decrease of the vein and ureter flow and oncometer.

*Conclusions.* — Digitalis causes a prompt and marked vasoconstriction in concentrations of 0.15 per cent for at least six hours after excision. Active samples of digitalin produce a distinct vaso-



constriction even in 2 per cent NaCl solution, in concentration as low as 1 : 50,000. Another sample of digitalin in 2 per cent NaCl was found practically ineffective in a concentration of 1 : 1000.

Robert obtained a marked constriction with digitalin in a concentration of 1 : 200,000, and Sakusoff with a dilation as great as 1 : 1,000,000.

11. *Ergot.* — The following perfusions were tried with two different fluid extracts, the activity of which on blood pressure had been proved on intact animals.<sup>5</sup>

**On kidneys. —**

Two perfusions of 2 per cent in diluted blood : negative.

One perfusion of 1 per cent in diluted blood : slight increase of vein and oncometer.

Two perfusions of 0.2 per cent in diluted blood : slight decrease of vein and oncometer in one, the other negative.

One perfusion of 0.08 per cent in diluted blood (warm) : negative.

**On legs. —**

One perfusion of 2 per cent in diluted blood : slight doubtful increase.

One perfusion of 2 per cent in 1 per cent NaCl : negative.

One perfusion of 0.4 per cent in 1 per cent NaCl : slight doubtful increase.

One perfusion of 0.08 per cent in 1 per cent NaCl : slight doubtful decrease.

**On intestines. —**

One perfusion of 0.2 per cent in diluted blood : negative.

One perfusion of 0.1 per cent in diluted blood : negative.

One perfusion of 0.16 per cent in diluted blood (warmed) : negative.

One perfusion of 0.12 per cent in diluted blood (warmed) : negative.

One perfusion of 0.03 per cent in diluted blood (warmed) : negative.

**On spleens. —**

One perfusion of 0.16 per cent in diluted blood (warmed) : slight increase of vein and decrease of oncometer.

Two perfusions of 0.08 per cent in diluted blood (warmed) ; one slight increase of vein, the other slight increase of oncometer.

*Conclusions.* — In most of the experiments the effect was quite negative, and in the others it was so small and variable that it may also be considered negative, although many of the perfusions were made under the best conditions. The vessels of these organs re-

<sup>5</sup> SOLLMANN and BROWN: *Journal of the American Medical Association*, 1905, xlv, p. 229.

sponded normally to such drugs as adrenalin, digitalis, chloral, and barium chloride. It must be concluded that ergot has no peripheral action on the vessels in the dog.

**12. Formaldehyde.** — Six perfusions of kidneys for forty minutes with 1 : 1000 solution in 2 per cent NaCl were entirely negative, although mercuric chloride was very effective on the same kidneys, either after (three cases) or before (one case) the formaldehyde.

**13. Hydrastinin hydrochloride.** — This was perfused through recently excised kidneys in the concentration of 1 : 5000, dissolved in diluted blood in two experiments, and in 1 per cent NaCl in four experiments. The blood perfusions were quite negative, and with the others the effects were so small and variable that they should also be classed as negative; if anything, they tended to decrease the vein flow and oncometer.

These negative results contradict the experiments of Bunge, who found marked increase in beef's kidneys with concentrations of 1 : 1,000,000 to 1 : 10,000.

**14. Hydrastis.** — A filtered 1 per cent solution of the fluid extract in 1 per cent NaCl was perfused through four kidneys. Three of these showed a fair increase of the vein and ureter flow, with a much lesser increase of the oncometer. The fourth kidney gave twice some decrease of the vein and ureter flow, with little change of the oncometer; this was followed by increase when the unpoisoned NaCl was resumed. It appears, therefore, that hydrastis produces some vasodilation, situated rather distally.

**15. Hydrocyanic acid.** — This was used in the proportion of 1 : 2500 in the following experiments:

On living kidneys, in diluted blood; five perfusions; all show a large increase of vein and ureter flow and oncometer.

On living kidneys, in 1 per cent NaCl; five perfusions; four showed the usual increase, one is not affected.

On kidneys injured by the previous perfusion of 0.3 per cent sodium fluoride for about an hour; three perfusions in diluted blood; one shows a good effect, the other two none. One perfusion in 1 per cent NaCl, fair effect.

On kidneys injured by the previous perfusion of 2 per cent NaCl for one to one and a half hours: Two perfusions in diluted blood: one fair effect, the other poor effect. One perfusion in 2 per cent NaCl; no effect.

On living kidneys in diluted blood containing 1.6 per cent NaCl; two perfusions; no effect.

On kidneys two days after excision; two perfusions in diluted blood; apparently some effect. Three perfusions in 1 per cent NaCl; no effect.

Two perfusions of a leg in 1 per cent NaCl appeared to show some increase of vein flow.

Three perfusions of a thyroid gland in 1 per cent NaCl produced no effect.

*Conclusions.* — The perfusion of a 1:2500 solution, in either blood or one 1 per cent NaCl, causes almost invariably a very marked increase of the vein and ureter flow and oncometer, when the perfusion is made through living kidneys. Injury by 0.3 per cent NaF or by 2 per cent NaCl generally abolishes the reaction. It is absent in dead kidneys. It is at once removed by adrenalin. The response of organs other than the kidneys needs further investigation.

**16. Juniper oil.** — Six perfusions were made with a saturated solution in 1 per cent NaCl. Four of these showed a marked increase of the vein and ureter flow, and two as marked a decrease. The changes in the oncometer were small, but tended in the same direction. Recovery occurred promptly. In five perfusions of a saturated solution in 2 per cent NaCl the results were quite negative.

The exact opposition of the results for the 1 per cent NaCl makes it unsafe to draw any conclusions; but in 2 per cent NaCl there are certainly no effects.

**17. Mercuric chloride.** — This was tried in 1:1000 solution in 2 per cent NaCl, in six perfusions. It caused in every case an immediate and conspicuous diminution of the vein flow, and practically arrested the ureter flow. There was no recovery after an hour's perfusion with unpoisoned NaCl solution. The oncometer was variable, being markedly decreased in two and slightly increased in two. In those cases in which it fell, it recovered under NaCl, and in one experiment it rose markedly above the normal. These effects also occurred after formaldehyde (1:1000).

There has evidently been an obstruction of the vessels, and it remains to decide whether this is due to coagulation of proteid within the lumen of the vessels, or to astringent action on the vascular walls, perhaps with loss of elasticity.

Several facts speak strongly against the view that it was due to intravascular coagulation. The effect occurred even when the

vessels had been flushed during five and one-half hours, so that scarcely any blood could have remained. A precipitate should be washed out during the hours' subsequent perfusion, so that recovery should occur. The oncometer would be markedly increased by obstruction through a precipitate. It must therefore be concluded that the action is due to the coagulation of the vessel walls and perhaps of the filtration membrane.

**18. Nitrite of sodium.**—A 1 : 1000 solution in 2 per cent NaCl was perfused through two kidneys for fifteen and thirty minutes. The effect on vein flow and oncometer was negative. (Sakusoff found dilation with 0.25 to 0.5 per cent.)

**19. Peptone-Witte.**—Two perfusions were made with a 1 : 100 solution in one per cent NaCl. The series is too small for definite conclusions, but the results were certainly not marked. The vein flow was unchanged, the ureter flow seemed somewhat increased, and the oncometer slightly decreased. (On beef's kidneys Thompson observed a definite but slight increase with concentrations of 0.08 and 0.33 per 1000.)

**20. Picric acid.**—This series comprises five perfusions of 1 in 10,000 of 2 per cent NaCl; eleven perfusions of 1 in 1000 of 2 per cent NaCl; and two perfusions of dead kidneys with 1 : 1000 of 1 per cent NaCl. In practically every case there was a very marked diminution of the vein flow and oncometer, and a still more conspicuous decrease of the ureter flow. The effect started promptly, but progressed slowly. The 1 : 10,000 solution produced very noticeable changes, but these were exaggerated when 1 : 1000 was substituted. The effects occurred after perfusion with sodium fluoride (1 : 1000). They were as conspicuous if the kidney had been flushed with the 2 per cent NaCl solution for hours as they were shortly after excision. There was little or no recovery when unpoisoned NaCl solution was perfused, even when the picric acid had only flowed for ten minutes.

*Conclusions.*—There is evidently an obstruction, the action being strictly analogous to that of mercuric chloride, which see.

**21. Saponin.**—Four perfusions were made with crude quillaja saponin (Merck), 1 : 10,000 of 2 per cent NaCl. These showed the same effects, namely, a fairly large *increase* of vein flow, a very considerable *decrease* of ureter flow, and very slight changes in the oncometer, — perhaps a tendency to increase. Succeeding perfusion with a 1 : 1000 solution had little further effect. There was but little recovery on perfusing unpoisoned NaCl solution.

These phenomena indicate a widening of the blood channels especially toward the proximal side, together with a lesser permeability of the glomerular capillaries to filtration.

22. **Sulfocyanide of sodium.**—This was negative in two perfusions with a 1:10,000 and in eight perfusions with a 1:1000 solution, both in 2 per cent NaCl.

#### DISCUSSION.

The principal results of these experiments are presented in schematic form in Table I.

With every drug, except saponin, the vein and ureter flow and oncometer vary in the same direction, indicating that the effects are essentially vascular, and emphasizing that the ureter flow in excised kidneys depends mainly upon the glomerular pressure. In every case the effects are essentially similar whether the perfusing fluid is blood or saline solution, which again points to a vascular rather than an epithelial action. However, the response is often weaker in the saline solution, and is more or less diminished by all injurious agencies. Two per cent sodium chloride practically abolishes the reaction to all but the most powerful poisons, such as saponin, arsenic, mercuric chloride, and picric acid. The last two, and perhaps also the arsenate of sodium, probably act by coagulating the protoplasm; but it is remarkable that formaldehyde has no effect.

The following poisons caused *vasodilation and increase of ureter flow*:

Chloral	Hydrocyanic acid
Hydrastis	Juniper <sup>6</sup>

The following caused *vasoconstriction and decrease of ureter flow*:

Adrenalin	Digitalis
Arsenate of sodium	Mercuric chloride
	Picric acid

The following were practically *ineffective* in the concentrations used:

Alcohol	Carbon dioxide	Formaldehyde <sup>6</sup>
Caffein	Carbon monoxide	Hydrastinin <sup>6</sup>
Cantharidin	Ergot <sup>6</sup>	Sulfocyanide of sodium.

<sup>6</sup> Indicates that the results are not quite conclusive.

TABLE I.  
SYNOPSIS OF THE RESULTS OF DRUG PERFUSIONS THROUGH EXCISED KIDNEYS.

Drug.	Concentration.	Fresh kidneys, perfusing fluid.				Dead Kidneys 1 per cent NaCl.	Remarks.
		Warm blood.	Cold blood.	1 per cent NaCl.	2 per cent NaCl.		
Adrenalin . . . . .	50,000	....	≡	≡	—	—	Similar results with excised leg, spleen and intestine
Alcohol . . . . .	1,000 100	....	....	0 0	0	....	Not enough data
Amyl nitrite . . . . .	1,500	....	....	....	≡	....	Not enough data
Arsenate of sodium . . . .	1,000	....	≡	....	0	....	Not enough data
Caffein citrated . . . . .	10,000	....	....	....	....	....	Not enough data
Caffein sodium benzoate . .	5,000	....	0	0	....	....	Not enough data
Cantharidin . . . . .	5,000	....	0	....	0	....	Not enough data
Carbon dioxide . . . . .	Saturated	....	0	....	....	....	Not enough data
Carbon monoxide and coal gas	Saturated	....	++++	....	....	....	Not enough data
Chloral . . . . .	1,000	....	....	....	....	....	Not enough data
Digitalin, sample A . . . .	50,000	....	....	....	≡	....	Not enough data
	10,000	....	....	....	≡	....	Not enough data
Digitalin, sample B . . . .	5,000	....	....	....	0	....	Not enough data
	1,000	....	....	....	0	....	Not enough data

[illegible]

The effects of adrenalin and digitalis are strictly comparable with those produced in living animals; the effects of picric acid and mercuric chloride would also presumably be identical if the same conditions could be reproduced. It is rather remarkable that the characteristic effects of arsenic, caffein, cantharidin, and juniper were not reproduced in the excised kidneys. It would seem, therefore, that these drugs, to produce these characteristic effects, either require the connection of the kidneys with the general circulation or central nervous system, or else that they do not act by modifying the renal circulation, but produce their effects directly on the renal epithelium. The actions of the other drugs on the blood vessels and kidneys of living animals are not sufficiently well ascertained to make a comparison with our results profitable.



## THE EFFECT OF VAGUS INHIBITION ON THE OUTPUT OF POTASSIUM FROM THE HEART.

BY W. H. HOWELL AND W. W. DUKE.

[From the Physiological Laboratory, Johns Hopkins University, Baltimore, U. S. A.]

IN a previous paper one of us has advanced the hypothesis that the vagus fibres of the heart "end in what may be designated as an inhibitory substance which, under the influence of the vagus impulses, is dissociated with the liberation of potassium compounds to which the phenomenon of inhibition is directly due."<sup>1</sup> To test this hypothesis, namely, that the phenomenon of vagus inhibition is at bottom a case of potassium arrest, the present authors have attempted to determine directly the influence of repeated stimulation of the vagus upon the potassium output in the mammalian heart. For this purpose the heart was isolated, in dogs, rabbits and cats, and kept beating upon a large supply of Locke's solution, fed to the heart through the coronary arteries under oxygen pressure. From time to time the circulation of the stock liquid was suspended and the heart was inhibited for one-half minute to a minute by stimulation of the vagus in the neck, or, as was frequently necessary, by stimulation of a branch from the level of the inferior cervical ganglion on the right side. At the end of the stimulation the heart was washed out through its coronary arteries by a special supply of the circulating liquid, amounting to 75 to 100 c.c. The vagus fibres were stimulated as often as they continued to give a visible inhibition of the auricles, that is, in the different experiments, from five to twenty times, and after each inhibition the heart was irrigated by the same special supply of circulating liquid. At the end of the experiment samples were taken of the circulating liquid which had passed once through the unstimulated heart, and of the special supply which had been circulated repeatedly through the heart after inhibition, and these samples were analyzed for their content in potassium. Control

<sup>1</sup> HOWELL, W. H.: This journal, 1906, xv, p. 280. See also MARTIN: This journal, 1904, xi, p. 370, and BOTAZZI: Archives de physiologie, 1896, p. 882.

experiments were made in which precisely the same procedure was followed, except that the heart was not stimulated, or was stimulated through the accelerator nerves, and samples for analysis were taken of the stock liquid which had passed once through the heart, and of the special supply which had been irrigated eight or more times through the heart. In determining the potassium in the samples taken for analysis the method proposed by Cameron and Failyer<sup>2</sup> was employed. The method consists in destroying the organic matter and driving off the ammonia by incineration, the conversion of the potassium and sodium salts into the corresponding chlorplatinate, the removal of the sodium chlorplatinate by washing with alcohol, and finally the solution of the remaining potassium chlorplatinate in hot water. From the latter salt a double iodide of platinum and potassium is formed by the addition of potassium iodide in acid solution. The double iodide gives a richly colored red-purple solution in which the amount of the potassium contained as chlorplatinate may be accurately determined by comparison in a colorimeter with standard solutions formed from known amounts of potassium chlorplatinate. The method is convenient and yields reliable quantitative results provided the following precautions are observed. 1. The process of incineration should be conducted in platinum crucibles or dishes. 2. In the conversion of the incinerated residue to chlorplatinate the addition of a large excess of platinum chloride must be avoided, and care must be taken in evaporating to dryness to use a low temperature. 3. In washing with alcohol, paper or asbestos filters should not be used; the washing must be conducted by centrifugalization and decantation.

Two series of experiments were made. In the first series of eight experiments the quantity of potassium was not determined by comparison with standard solutions, but the relative amounts of potassium were estimated in the colorimeter by comparison of the solution which had passed once through the unstimulated heart with the solution which had been irrigated repeatedly through the inhibited heart. In every case it was found that the latter solution contained a larger amount of potassium. In five control experiments in which the inhibitory nerves were not stimulated a similar increase was not obtained. The solutions prepared from the two

<sup>2</sup> See especially SCHREINER and FAILYER, Bulletin 31, U. S. Department of Agriculture, 1906.

samples were either identical in color, or varied so slightly in one direction or the other, that the variation fell within the possible limits of error of the method. In a second series of seven experiments quantitative estimations of the amount of potassium were made by comparison with standard solutions obtained from a carefully prepared specimen of potassium chlorplatinate.<sup>3</sup> The results of these analyses are given in the following table. In most cases

Date.	Number of stimulations.	Quantity of liquid used.	Amount of potassium in mgrm. for each c.c.		Increase in potassium in each c.c. of the circulated liquid after vagus inhibition.	Estimated total increase in potassium (?) <sup>2</sup>
			Without stimulation.	With stimulation.		
June 3	11 <sup>1</sup> (?)	c.c. 70	0.155	0.180	0.025 or 16 per cent	mgrm. 1.75
June 7	7	90	0.133	0.156	0.023 or 17 " "	2.07
Oct. 1	7	75	0.139	0.166	0.027 or 19 " "	2.03
Oct. 6	7	45	0.128	0.160	0.032 or 25 " "	1.44
Oct. 12	5	...	0.225	0.250	0.025 or 11 " "	...
Oct. 19	6	60	0.197	0.242	0.045 or 23 " "	2.70
Oct. 26	8	80	0.129	0.167	0.038 or 29 " "	3.04

<sup>1</sup> The heart was stimulated eleven times through the vagus, but it is probable that some of the later stimulations were ineffective.

<sup>2</sup> The estimate of the total output of potassium can only be considered as approximate. In most of the experiments there was a certain amount of leakage, so that it was impossible to collect the entire amount irrigated through the heart. Moreover it was impossible to prevent a certain amount of dilution of the special supply, since after each irrigation a small amount was left in the heart to be washed away when the stock solution was turned on, and vice versa. Undoubtedly in every experiment only a portion of the increased output of potassium was recovered.

the analyses were made in duplicate with closely concordant results. In contrast with these figures are the results obtained from three control experiments in which the heart was irrigated repeatedly with the same small supply of liquid, but without stimulation of the vagus. The procedure was exactly the same as in the experiments with vagus stimulation. One of these controls was carried

<sup>3</sup> This specimen was kindly furnished by Prof. J. H. KASTLE of the U. S. Public Health and Marine Hospital Service, Washington, D. C.

out after a vagus experiment had been performed (June 7), and one before a vagus experiment (October 26).

## CONTROL EXPERIMENTS

Date.	Number of circulations.	Quantity of liquid circulated.	Amount of potassium in milligrams.		Increase of potassium in each c.c. of the circulating liquid.
			After one circulation.	After eight circulations.	
June 7	8	c.c. ...	0.166	0.170	0.004 or 2.4 per cent
Oct. 1	8	75	0.143	0.143	.....
Oct. 26	8	80	0.129	0.137	0.008 or 6.2 per cent

In the experiment of June 7, the previous vagus stimulation had given an increase of 0.023 mgm. per c.c. or 17 per cent, while in the experiment of October 26, the subsequent vagus stimulation gave an increase of 0.038 mgm. per c.c. or 29 per cent. These results corroborate, therefore, the previous series of five controls in which the accelerator nerves alone were stimulated and in which there was no distinct variation in the potassium contents of the circulated liquid.

It seems evident from these experiments that stimulation of the vagus nerve in the mammalian heart causes a liberation of potassium from the heart substance in a diffusible form. Our experiments have indicated, moreover, that this output of potassium occurs in the auricular end of the heart. This conclusion was forced on us first by the fact that in the rabbit's heart the vagus nerve, in the isolated heart, retains its inhibitory action much longer than in the dog's heart, so that as many as twenty successive inhibitions of the whole heart may be obtained for the rabbit while seven or eight only can be obtained for the dog, and of these only two or three affect the ventricle as well as the auricle. Nevertheless in the single experiment made upon the rabbit, the increase in potassium was very small, a fact which was attributed to the small mass of auricular tissue in this animal compared with the large volume of circulated liquid. In the dog, moreover, only the first two or three stimulations caused stoppage of the ventricle as well as the auricle, the succeeding stimuli, applied as a rule to a branch passing from

the inferior cervical ganglion, to the heart, on the right side, inhibited only the auricles. In these cases, however, the increase in potassium was well marked. The failure of the vagus impulses in these latter cases to stop the ventricle also is to be attributed to the ready assumption of an independent rhythm by the ventricle, when the heart beat is maintained by irrigation with a solution of inorganic salts. We have had frequent opportunities of noticing this fact. Our experience is in accord with the general belief that the vagus impulses affect directly only or mainly the auricular end of the heart. When we remember that the auricular tissue weighs but a few grams, it is apparent that a liberation of as much as 0.4 to 0.5 mgm. of K at each stimulus, within the substance of the auricular tissue itself, is quite sufficient to bring on a condition of potassium inhibition. For the reasons stated in the footnote to Table I, it is more than probable that our method did not enable us to recover all the potassium liberated by the vagus stimulation, and our figures are therefore minimal rather than maximal estimates of the amount of potassium set free in diffusible form at each stimulation. Experiments on the isolated heart show that when the content of the circulating liquid in potassium approaches 0.05 per cent, or even less, the heart ceases to beat, and our experiments indicate that such a concentration of potassium may occur in the auricular tissue, or in certain parts of it, during vagus stimulation. In the paper previously referred to and in a subsequent paper by the present authors,<sup>4</sup> it was shown that the inhibitory action of the vagus nerve on the heart is markedly favored by an increase in the potassium contents of the circulating liquid, while on the other hand it is markedly diminished by the circulation of a liquid containing no potassium, or indeed may fail altogether under this last condition. Taking these facts in connection with the result obtained in this paper, the authors feel that the theoretical view here advanced, namely, that the vagus stops the heart by causing the liberation of diffusible potassium from some indiffusible compound normally present in the heart tissue, has a convincing amount of evidence in its favor. The view commonly taught of the nature of vagus inhibition, namely, that it is due to the setting up of synthetic or assimilatory processes within the heart substance, while it is conceivable, has little or nothing in the nature of positive evidence to support it. The energy transmitted

<sup>4</sup> HOWELL and DUKE, *Journal of Physiology*, 1906, xxxv, 131.

as a nerve impulse cannot, so far as our actual knowledge goes, be utilized to perform the chemical work of synthesis. This function is fulfilled most probably by the heat energy liberated by the oxidative changes in the tissue, and such changes are characteristic of the nerve impulses which excite functional catabolism rather than of the inhibitory impulses. We conceive that the inhibitory impulses, like the motor or excitatory impulses, act as a liberating stimulus which sets up changes of dissociation, but this dissociation is of such a character that diffusible potassium is set free and through its action the functional catabolism is inhibited.

The authors' conclusion that the increase in potassium in the circulating liquid observed after stimulation of the vagus is due to an increased output of potassium from the heart may be criticised upon several grounds. So far as we can see the following possible objections might be made. The increase may have been due to evaporation occurring in the repeated transfer of the liquid from one receiver to another; it may have resulted from diffusion with the liquids of the heart tissue possibly richer in diffusible potassium than the circulating liquid, or it may have occurred because the action of the vagus fibres caused an absorption of water.<sup>5</sup> Each of these explanations on examination is found to be insufficient to account for the results obtained. To determine the effect of evaporation alone several determinations of the specific gravity of the liquid used were made at the beginning and the end of an experiment. The determinations were made with a pycnometer and gave such results as the following. Experiment of June 7:

Weight of flask filled with stock liquid . . . . .	26.6877 gm.
Weight of flask alone . . . . .	7.8366 "
Weight of liquid . . . . .	18.8511 "
Weight of flask filled with liquid after eight circulations . . . . .	26.6900 "
Weight of flask . . . . .	7.8366 "
Weight of liquid . . . . .	18.8534 "

The gain in weight of this volume of liquid, as a result of eight circulations through the heart was therefore 2.3 mgm. Since the capacity of the pycnometer was equal to 18.7 c.c., the total increase for the 90 c.c. used in this experiment was equal to

<sup>5</sup> This last suggestion was made to the authors by Professor MENDEL.



$90/18.7 \times 2.3$  or 11 mgm. If we assume that this increase was due solely to evaporation, the proportional increase in the potassium would be equal to 0.138 mgm., since in the mixture used the potassium chloride constituted 2.3 per cent of the total solid constituents. As a matter of fact the total increase in potassium found in this experiment was equal at least to 2.07 mgm. Similar results were obtained in the other experiments in which the pyknometer was used. With regard to the second objection, namely, that the circulating liquid was enriched with potassium by diffusion from the heart tissue, it is to be remembered in the first place that the heart was always perfused with a litre or more of the circulating liquid before the experiment upon stimulation of the vagus nerve was begun. It is probable, therefore, that by the time that the special supply was turned into the heart, an equilibrium had been established between the circulating liquid used and the heart liquids, in regard to the easily diffusible constituents. Moreover, in the control experiments in which the heart was perfused a number of times with a small supply of the circulating liquid without stimulation of the vagus nerve, no such increase in potassium was observed. In some of these experiments the quantity of potassium was unchanged; in some it showed a slight decrease, and in others a slight increase, the maximal result of this latter kind being an increase of 6.2 per cent, whereas in the vagus experiment on the same heart the increase was equal, at the least, to 29 per cent. With regard to the third objection, namely, that the vagus stimulation may cause an absorption of water and thereby increase the percentage of potassium, the pyknometer determinations, referred to above, are perhaps sufficient to show that such an explanation of the results obtained is entirely inadequate. The circulating liquid, in the experiment of June 7, for example, contained 1.07 gm. of material to each 100 c.c. The potassium in this liquid was increased by 17 per cent as the result of vagus stimulation. If we assume that this increase was due to an absorption of water which should affect alike the concentration of all the substances in solution, then the total increase for these solids would have been from 1.07 to 1.252 gm., that is, an increase of 182 mgm. to each 100 c.c. or 1.82 mgm. to each c.c. According to the pyknometer determinations, however, the total increase in concentration for each c.c. was equal only to 0.123 mgm. Moreover quantitative estimations of some of the other constituents in the solution showed

conclusively, that there was no such increase in concentration,\* as must have followed, if there had been sufficient absorption of water to raise the concentration of potassium to 17 per cent or more. As stated below, the dextrose showed always a small decrease in concentration, and the calcium, so far as could be determined by the method used, was not changed.

**The effect of vagus inhibition on the calcium contents of the circulating liquid.** — It was our original intention in planning this series of experiments to determine both the potassium and the calcium contents of the circulating liquid after stimulation of the vagus and of the accelerator. The method used for determining the calcium, which is described briefly below, did not, however, prove to be sufficiently sensitive for our purpose. The minimal variation which could be detected with certainty amounted to 15 to 20 per cent. Within this limit no change in the calcium could be detected as a result of vagus inhibition, but the authors propose to continue this part of the work both for the accelerator and vagus nerves, making use of a more delicate method for the determination of the calcium.

#### METHODS.

**The operation for isolating the heart.** — The anesthetized animal was tracheotomized and connected with an ether bottle. The operation was then performed in the following steps: the upper portion of the sternum and the adjacent first ribs were resected and the internal mammary arteries were ligated; the ribs on both sides were cut away so as to open freely the thoracic cavity; the brachio-cephalic artery was exposed and a cannula was inserted into it to be used subsequently for the inflow of the circulating liquid; the left subclavian artery was ligated at its origin from the aorta, and a ligature ready for tying was laid round the aorta above the origin of the first intercostal arteries; the aorta was cut below this last ligature and while the animal was bleeding the supply of circulating (Locke's) liquid was turned on under moderate pressure through the cannula placed in the brachio-cephalic artery; after sufficient time had been allowed for the washing out of the blood in the heart and aorta, the ligature round the aorta was tied, and thenceforward the supply of circulating liquid passed through the coronary arteries alone; the inferior vena cava was then opened widely to give an outlet to the coronary circulation and the azygos vein and the superior cava were ligated. To prevent an outflow from the pulmonary artery into the lungs, stout ligatures were tied round the roots of the lungs close to the heart. Finally the pericardium was opened along the ventral surface of the heart, and for convenience' sake the permanent outflow cannula was inserted into the superior vena cava, the inferior cava



being ligated close to the heart. The outflow cannula was thrust down far enough to lie within the right ventricle, and it was provided with lateral openings at the level of the right auricle. After the completion of these operations the vagus, or the accelerator nerve, was prepared for stimulation, while the heart was beating upon the artificial circulation. This method of isolating the heart succeeds in practically all cases with dogs, cats or rabbits, the only animals used in these experiments. With the circulating liquid employed, the isolated heart beats very well indeed for a number of hours, provided the aortic valves prove competent for the pressure used, and protect the left auricle and ventricle from over-distension.

**The circulating liquid.**—As in our former experiments the circulating liquid was made up approximately according to the following formula.

NaCl . . . . .	0.9	per cent
KCl . . . . .	0.025	" "
CaCl <sub>2</sub> . . . . .	0.023	" "
NaHCO <sub>3</sub> . . . . .	0.02	" "
Dextrose . . . . .	0.10	" "

In some cases the amount of calcium chloride was increased, and in some of the experiments the amount of potassium chloride was varied. The circulating liquid was saturated with oxygen and was driven into the heart under oxygen pressure. The pressure under which the liquid entered the heart was determined by a mercury manometer attached to the inflow tube. Experience showed that the heart beat best when not fed under too high a pressure. Instead of imitating the normal aortic pressure of 100 to 150 mm. mercury, the circulation was usually maintained under a pressure of from 40 to 60 mm. of mercury. An adequate supply of oxygen in the circulating liquid seems to be quite essential for a successful experiment with the mammalian heart.

**Conduct of an experiment.**—The circulating liquid was contained in two reservoirs both of which were kept immersed in a large tank of water maintained at a constant temperature of 37° C. One reservoir was large, holding about eight litres, and served as the stock solution on which the heart was kept beating, except during the short periods in which the nerves were being stimulated. The other reservoir was small with a maximum capacity of 250 c.c. The liquid in this reservoir, usually from 75 to 100 c.c., was run through the heart after each stimulation of the nerve. The method of procedure was as follows. When the nerve (vagus or accelerator) was to be stimulated, the supply of circulating liquid was shut off, and the heart was allowed to drain itself as far as possible. The beat of the heart was not interfered with by this temporary cessation of the circulation. After draining for a few seconds the nerve was stimulated, usually for a minute, and then the circulating liquid from the small reservoir was run through the heart, and the outflow was caught in a

special receiver. After the heart had been washed out by this supply, it was again allowed to drain for a few moments, and then the stock supply was turned on and continued until the next stimulation of the nerve. In this way the special supply of liquid in the small receiver was run through the heart a number of times, five to twenty. At the end of the experiment samples of this supply, and of the stock liquid which had been passed once through the heart were taken for analysis. It may be stated that the circulating liquid remained water clear, no matter how often it had been passed through the heart, provided the latter had been thoroughly washed out in the beginning of the experiment. It contained, however, a minute amount of protein material, as shown by the fact that a slight opalescence developed on boiling. It was noteworthy also that liquid which had passed through the heart one or more times developed bacteria readily upon standing in a warm room, whereas the original liquid remained clear.

**Stimulation of the nerve.** — The vagus nerve was stimulated at first in the neck. In all cases the effect for the first two or three stimulations was a distinct inhibition of the heart, the inhibition in fact was complete except in the case of the cat. With rabbits this portion of the vagus continued to give complete inhibition of the heart for a couple of hours, during which it was stimulated as many as twenty times. In the dog, however, the cervical portion of the vagus lost its effect after two or three stimulations. If the electrodes were then applied to a branch springing from the inferior cervical ganglion on the right side, it was usually possible to obtain five or six additional inhibitions which affected the whole heart or the auricles alone. Stimulation of the nerve below the inferior cervical ganglion on the left side was ineffective after the cervical portion of the vagus had ceased to respond.

**Methods of analysis.** — The solutions circulated through the heart were analyzed for their contents in potassium and calcium, and in some cases also for the dextrose. For the latter substance Pavy's method was used and gave excellent results. As stated above the solution, after it had passed through the heart one or more times, developed bacteria quite rapidly and suffered in consequence a progressive loss of its sugar. In making the determinations for dextrose, it was necessary, therefore, to examine the solutions immediately at the end of the experiment, or if this was not possible to sterilize the samples by boiling in flasks with a plug of cotton. It was found without exception that the liquid circulated through the heart suffered a slight loss of dextrose. In those cases in which a small supply of the circulating liquid (200 c.c.) was passed through the heart a number of times (6 to 9) the diminution in dextrose was equivalent to 0.5 to 0.6 c.c. of the standard solution used, that is, there was a loss of sugar amounting to 20 to 30 mgm. The analyses for sugar were made only in the earlier experiments, and no data were obtained to indicate whether or not the loss of sugar was specifically influenced by stimulation of the inhibitory or the accelerator nerves.

For the determination of the potassium the authors made use of the colorimetric method described by Cameron and Failyer.<sup>6</sup> The method involves the formation of a chlorplatinate of potassium and the development from this of a double iodide having a red color by the addition of potassium iodide. The method in detail is as follows. Two cubic centimetres of the solution to be examined were placed in a platinum crucible and  $\frac{1}{2}$  c.c. of dilute sulphuric acid (1 to 4) was added. The solution was evaporated to dryness over the water bath, heated over a sand bath until the excess of sulphuric acid was driven off, and was then incinerated over a Bunsen burner at a dull red heat. The ash was moistened with a few drops (10) of hydrochloric acid (acid 1 part, water 1 part) and a few drops (4) of a solution of platinum chloride (1.73 gm. platinum chloride to 25 c.c. water). This solution was slowly evaporated nearly to dryness over the water bath, and the evaporation was then carried just to dryness at a lower temperature. The residue was then washed thoroughly with 95 per cent alcohol by decantation and centrifugalization as follows. Four or five c.c. of alcohol were poured on the residue in the crucible, and the material was carefully rubbed with a glass rod and then decanted into a centrifugalizing tube. Part of the insoluble residue passed into the tube, while a part remained in the crucible. This process of washing was repeated five times, and the crucible containing a portion of the residue was dried over the water bath. The centrifugal tube was placed in the centrifuge for a few minutes, the precipitate being so heavy that a few minutes centrifugalizing enabled one to pour off the supernatant alcohol without disturbing the residue. The latter was then washed several times with a few c.c. of alcohol by centrifugalizing and decanting, and the washed residue was dried over the water bath. The residues in the tube and in the crucible were then dissolved in hot water, enough being used to make a solution of 25 to 50 c.c. After cooling the color was developed in this solution by first making acid with hydrochloric acid, and then adding  $\frac{1}{2}$  c.c. of a 25 per cent solution of potassium iodide. The red color which resulted increased gradually to a maximum, and it was found safer to allow the solution to stand ten to twelve hours before making a colorimetric determination. In making this determination the red colored solution was diluted to 100 c.c. so that the original solution, so far as its potassium was concerned, was diluted fifty times. The colored solutions thus obtained from specimens of the circulating liquid, with and without stimulation of the nerves, were compared with each other, and with standard colored solutions obtained from known weights of potassium chlorplatinate. In making the colorimetric determinations the colorimeter devised by Schreiner<sup>7</sup> was used. It was found to give more satisfactory results than the Duboscq colorimeter.

<sup>6</sup> Journal of the American Chemical Society, xxv, 1063, 1903. See especially SCHREINER and FAILYER, Bulletin 31, U. S. Department of Agriculture, 1906, p. 31.

<sup>7</sup> Bulletin 31, U. S. Department of Agriculture, 1906.

The method of procedure given above differs from that described by Schreiner and Failyer in two points, both of which, according to our experiments, are of fundamental importance. In the first place the precipitate of chlorplatinates was washed with alcohol by decantation and centrifugalization. We have found that it is impossible in this process to use filter paper or the asbestos filters recommended by these authors, owing to the fact that some of the platinum chloride adheres so firmly to the filters that it is not removed by the alcohol. Analyses of solutions containing a known amount of potassium salt, when the washing with alcohol was effected through filtration, gave always results that were much too high. It does not seem at all probable that this retention of the platinum salt was due to the presence of ammonia in the filters, since the paper filters were carefully washed and the asbestos was heated to redness in a platinum crucible before using. A similar difficulty was encountered in the use of porcelain crucibles. When the process of incineration and formation of the chlorplatinates was carried out in a porcelain crucible, the quantitative result was invariably too high a yield of potassium as determined by the colorimetric method. Experiments indicated that this result was due to the fact that in washing the residue with alcohol, some of the alcoholic solution of the excess of platinum chloride present adhered firmly to the porcelain surface and was not washed out even by a relatively large excess of alcohol. If the glaze of the crucible was imperfect the error due to this fact was much increased, but it might occur also with porcelain crucibles of the best quality in which the glazed surface was intact. With the alterations in the method that we have described, namely, the use of platinum crucibles exclusively, the avoidance of filters in the process of washing with alcohol, and care in not using too high a temperature in the evaporation of the solution of chlorplatinates, the method has given very satisfactory quantitative results when applied to solutions, such as Ringer mixtures, containing known amounts of potassium. The methods used for determining the calcium in the circulating liquid were not so certain and reliable as in the case of the potassium. We employed the turbidity method described by Schreiner and Failyer.<sup>8</sup> The method consists in precipitating the dilute solutions of calcium as oxalate and then determining the relative amount of the precipitates by comparing the turbidity of the solutions with the aid of a colorimeter. The authors modified the method to the extent of converting it into a colorimetric turbidity comparison. They found it impossible at times to compare satisfactorily the depth of turbidity alone, but by covering the reflector with a red purple paper the two columns of liquid to be compared were given a reddish tint, the depth of color being less in the tube containing the denser precipitate. By this means we obtained much more accurate comparisons, but at times it also proved unsatisfactory owing to some slight difference in tint which made an exact matching of the tubes impossible.

<sup>8</sup> *Loc. cit.*

As stated above the results obtained by this method were not satisfactory, and it is proposed to continue this side of the work with the employment of a more sensitive method for determining the calcium.

SUMMARY.

1. When an isolated mammalian heart is kept beating upon an artificial circulation of Locke's liquid, stimulation of the vagus nerve causes an increase in the potassium contents of the circulating medium. Under the conditions of the experiment, namely, repeated circulations of a small supply of the liquid after maximal stimulation of the vagus, the increase in the potassium may amount to as much as 29 per cent. It is believed that this increase is referable to an output of potassium from the heart substance, due, probably, to the fact that the inhibitory nerve impulses cause a dissociation of an indiffusible compound in the heart substance and the liberation of the potassium in diffusible form. It is estimated that each stimulus (one half to one minute) may liberate between 0.4 and 0.5 mgm. of potassium. Assuming that the process occurs in the auricular tissue or in a definite portion of the auricles, this amount of potassium should be sufficient to inhibit the heart. The results obtained are presented, therefore, as evidence in favor of the view that the inhibitory action of the vagus nerve upon the heart is mediated through the influence of diffusible potassium compounds set free in the heart by the inhibitory impulses.

2. Stimulation of the vagus nerve causes no detectible change, within the limits of delicacy of the method employed, in the calcium contents of the circulating liquid.

3. Stimulation of the accelerator nerve causes no increase in the potassium contents of the circulating liquid.

## CHEMICAL STUDIES ON GROWTH. — IV. THE TRANSFORMATION OF GLYCOGEN BY THE ENZYMES OF EMBRYONIC TISSUES.<sup>1</sup>

BY LAFAYETTE B. MENDEL AND TADASU SAIKI.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE absence of glycogen from the liver of embryo pigs ranging in size from 85 to 230 mm., which was reported in an earlier paper,<sup>2</sup> has suggested the inquiry whether the embryo liver is capable of inducing the transformations of glycogen characteristic for the adult organ. We are not concerned with the question whether the post-mortem conversion of glycogen is in any respect related to the intra-vital functional alteration of this carbohydrate in the liver. Since an abundance of evidence is now available to show that the disappearance of glycogen is associated with a specific enzymatic reaction in which the activity of an hepatic amylase (glycogenase) is demonstrable, the theory of a "vital" cellular activity to account for the familiar phenomenon has lost its merit.<sup>3</sup> It is certain, at any rate, that the liver contains an enzyme capable of changing both deposited and foreign (added) glycogen into sugar. The probable significance of enzymes, *i. e.*, intracellular enzymes, in the chemical working of active living tissues has particularly been emphasized by Hofmeister.<sup>4</sup> Is a glycogen-converting enzyme formed or available in the *embryo* liver at a period when the glycogenic function has apparently not yet been called into use? To this inquiry we have directed experimental observations.

The liver of the embryo pig is rich in blood which cannot satis-

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> MENDEL and LEAVENWORTH: This journal, 1907, xx, p. 123.

<sup>3</sup> This topic is discussed with comprehensive references to the literature of the subject by PICK (F.): *Beiträge zur chemischen Physiologie*, 1903, iii, p. 163.

<sup>4</sup> Cf. HOFMEISTER: *Die chemische Organisation der Zelle*, 1901.

factorily be removed from the tissue as in the case of larger organs. In examining the embryo liver for enzymes the participation of the blood in any of the reactions observed must be taken into account. The blood is known to contain an amyolytic enzyme, or enzymes, and we have therefore compared its amyolytic activity in the embryo with that of the liver, the developing muscular tissue also being studied for contrast.

**Method.** — The embryonic material (from the pig) was obtained fresh, comminuted finely, and allowed to stand under several volumes of alcohol during one to two days. The residue was then filtered off on a hardened paper, washed with alcohol, pressed as dry as possible, and finally dried in a high vacuum desiccator.<sup>5</sup> It was then finely pulverized. The experiments were conducted by allowing

0.5 gm. tissue powder,  
20 c.c. 2 per cent glycogen solution,  
20 c.c. water,  
Toluene,

to digest at 23–25° C. during forty-eight hours. The mixture was then made up to 50 c.c., filtered, and the reducing power determined in 20 c.c. of the filtrate by the Allihn gravimetric method. In a few experiments, where smaller quantities of tissue were unavoidably employed, 10 c.c. water and 10 c.c. 2 per cent glycogen solution with toluene were used, and the mixtures made up to 25 c.c. before filtering. The results are expressed in the table in terms of CuO. The figures calculated for 100 gm. of fresh material have only comparative value, of course; for the reducing power is not strictly proportional to changes in sugar content. Inasmuch as the nature of the sugar here present is not definitely known and we were searching for the marked variations in amyolytic power, no attempt has been made to express the results in terms of sugar formed. In every case control experiments were conducted with comparable boiled mixtures of tissue and glycogen solution and an allowance was made for the reduction (usually very slight) directly attributable to the materials entering into the digestive mixtures. The data are summarized in tabular form on page 66.

**Discussion of the results.** — The value of the data here reported is dependent upon the assumption that the glycogen-converting prop-

<sup>5</sup> BENEDICT and MANNING: *American chemical journal*, 1902, xxvii, p. 340;  
GORE: *Journal of the American Chemical Society*, 1906, xxviii, p. 834.



Size of embryo. <sup>1</sup>	Tissue used.	No. of embryos used.	Wt. of tissues.		Residue in 100 gm. fresh tissue.	Dry tissue in digestion.	Comparative glycogen-digesting power expressed as CuO, in gm.			
			Fresh.	Dried.			From un-boiled digestion.	From boiled digestion.	Difference between A and B.	Calculated from C for 100 gm. fresh tissue.
mm.			gm.	gm.	gm.	gm.	A	B	C	average
30	Liver	8	3.5	0.7	20.0	0.20	0.0104	0.0004	0.0100	1.222
37	Liver	9	5.2	0.6	11.5	0.18	0.0064	0.0003	0.0061	0.487
40	Liver	21	19.0	3.0	16.0	0.50	0.0888	0.0004	0.0884	0.672
30	Muscle	20	17.2	1.0	5.8	0.50	0.0485	0.0002	0.0483	1.401
63	Liver	..	23.9	3.9	16.2	0.50	0.0885	0.0012	0.0073	0.591
63	Muscle	..	24.5	1.4	5.7	0.50	0.0559	0.0032	0.0527	1.502
63	Muscle	7	10.0	0.6	6.1	0.25	0.0462	0.0007	0.0455	1.387
100	Liver	9	39.5	5.4	13.8	0.50	0.0102	0.0007	0.0095	0.655
100	Muscle	9	11.0	0.7	6.4	0.25	0.0550	0.0024	0.0526	1.683
125	Liver	..	18.6	4.1	21.0	0.50	0.0152	0.0015	0.0137	1.438
125	Liver	4	24.0	3.3	13.7	0.50	0.0120	0.0004	0.0116	0.795
125	Muscle	..	37.0	2.4	6.6	0.50	0.0495	0.0085	0.0410	1.353
125	Muscle	9	12.5	0.8	6.5	0.25	0.0554	trace	0.0554	1.801
188	Liver	4	43.0	6.3	14.7	0.50	0.0275	none	0.0275	2.021
188	Liver	5	61.5	9.5	15.4	0.50	0.0116	none	0.0116	0.893
188	Muscle	4	29.8	2.2	7.3	0.50	0.0518	0.0041	0.0477	1.741
225	Liver	3	54.0	7.0	13.0	0.50	0.0340	none	0.0340	2.210
225	Liver	3	48.0	7.9	16.5	0.50	0.0470	0.0012	0.0458	3.778
225	Muscle	..	45.4	4.3	9.4	0.50	0.0818	0.0091	0.0727	3.417
225	Blood	..	15.0	1.15	7.7	0.50	0.0401	0.0006	0.0395	1.521
275	Liver	1	31.5	5.3	16.7	0.50	0.0972	0.0158	0.0814	6.797
275	Liver	1	37.0	6.4	17.2	0.50	0.0817	0.0234	0.0583	5.014
275	Muscle	1	18.5	3.2	17.2	0.50	0.0670	0.0040	0.0630	5.418
275	Muscle	1	23.0	3.5	15.4	0.50	0.0693	0.0116	0.0577	4.443
275	Blood	1	21.5	2.9	13.3	0.50	0.0458	0.0059	0.0399	2.653
275	Blood	1	21.0	2.8	13.3	0.50	0.0519	0.0036	0.0483	3.212
Adult pig	Liver	1	81.0	15.4	19.0	0.50	0.0469	0.0128	0.0341	3.239
Adult pig	Liver	1	78.0	21.3	27.3	0.50	0.0393	0.0070	0.0323	4.409
									0.0033	3.824

<sup>1</sup> Cf. This journal, 1907, xx, p. 90.

erties of tissues are not destroyed by alcohol. This is in entire accord with the recent observations of Schöndorff and Victorow,<sup>6</sup> who have found that glycogen does not disappear in a liver kept under alcohol, and that the amylolytic enzyme is preserved, recovering its activity when the alcohol is removed. Many older studies have indicated the applicability of the method of study here employed.<sup>7</sup>

The reducing power (*i. e.*, sugar content) of the *boiled* control (enzyme-free) tissue extracts was in general very slight. The fact that the higher values were usually obtained with the muscular tissue may be referable to its higher content of inherent carbohydrate. The low figures for some of the active digestions are not to be explained by any lack of digestible glycogen, since this was always present in considerable excess in each digestion. Data from adult livers are given for comparison.

The amylolytic power of the embryonic blood has, in each case examined, been smaller than that of corresponding liver or muscle tissue. This deserves emphasis because the embryonic liver is filled with blood which cannot be satisfactorily removed therefrom. The glycogen-converting power of muscle and liver is therefore not referable solely to the blood which they contain. It is the more important because some physiologists, notably Röhmman and his pupils, have maintained that the transformation of glycogen is primarily due to the activity of the tissue fluids (blood, lymph) rather than the enzymes of the cells themselves. F. Pick's<sup>8</sup> experiments on adult tissues gave the following comparative data regarding amylolytic power:

100 gm. blood digested	0.31 gm. glycogen in three hours.
100 gm. liver digested	0.69 gm.     "     "     "     "
100 gm. kidney digested	2.37 gm.     "     "     "     "

Borchardt<sup>9</sup> believes that the liver and blood enzymes are identical, but adds that "the activity of the ferment is greater in the liver than in the blood." Pugliese and Domenichini<sup>10</sup> have lately main-

<sup>6</sup> SCHÖNDORFF and VICTOROW: *Archiv für die gesammte Physiologie*, 1907, cxvi, p. 495.

<sup>7</sup> Cf. BAINBRIDGE and BEDDARD: *Bio-chemical journal*, 1907, ii, p. 89.

<sup>8</sup> PICK (F.): *Beiträge zur chemischen Physiologie*, 1903, iii, p. 174.

<sup>9</sup> BORCHARDT: *Archiv für die gesammte Physiologie*, 1903, c, p. 259.

<sup>10</sup> PUGLIESE and DOMENICHINI: *Archives italiennes de biologie*, 1907, xlvii, p. 1.

tained that the amylolytic enzyme is produced by the liver and is poured out from that organ into the circulation. New-born dogs and cats possess a feeble amylolytic power in both liver and blood at the time of birth; the enzyme content is said to increase with age more rapidly in the liver than in the blood.

In our own experiments the developing muscle tissue (which contains glycogen during embryonic life<sup>11</sup>) gave evidence, during the earlier stages of embryonic life, of a relatively greater glycogen-digesting power than that of liver tissue (free from glycogen). The amylolytic power of the embryonic liver tissue tends, however, to increase with the age of the embryo, ultimately overtaking that of the muscular tissue which shows a similar progressive tendency. Loewi<sup>12</sup> has found that the amylolytic power of the blood of different individuals of the same sort is approximately constant, although groups or species vary widely from each other. Nutrition cannot account for such marked distinctions. Loewi calls attention to the variations associated with age, without attempting any explanation. Thus the full-grown dog furnished more active blood than the puppy. For the adult muscle tissue Kisch<sup>13</sup> has shown that the amylolytic power is not dependent upon the nutritive or functional condition of the animal, the regulation of glycogen conversion apparently being governed by some other factor than the demand of the organism for sugar.

The principal feature to be emphasized in the present experiments is the variable equipment of amylolytic enzymes in the embryonic tissues and its correlation with developmental changes. The embryo liver, which is early free from glycogen, only gradually acquires its characteristic digestive capacity, the amylolytic efficiency increasing with growth.

<sup>11</sup> Cf. This journal, 1907, xx, p. 124.

<sup>12</sup> LOEWI: Sitzungsberichte der Gesellschaft zur Beförderung der Naturwissenschaften zu Marburg, 1904, No. 8.

<sup>13</sup> KISCH: Beiträge zur chemischen Physiologie, 1906, viii, p. 210.

## CHEMICAL STUDIES ON GROWTH.—V. THE AUTOLYSIS OF EMBRYONIC TISSUES.<sup>1</sup>

BY LAFAYETTE B. MENDEL AND CHARLES S. LEAVENWORTH.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

IN attempting to elucidate the chemical mechanism of nutrition considerable attention has been directed in recent years to the disintegrative changes which can occur in individual tissues. These studies have shown that autolytic processes may easily be observed in various organs removed from the body; but the real significance and importance of such processes of self-digestion has been debated by different investigators. One group insists that autolytic disintegration is a normal occurrence in living as well as surviving tissues; while other physiologists incline to the view that autolysis is distinctly a post-mortem phenomenon and takes no part in the intermediary processes of tissue metabolism.<sup>2</sup> Whether we accept the conclusion that autolytic degradation plays a normal rôle in the living animal or not, there can be no question that different tissues are variously equipped with potential capacities in the form of more or less specific enzymes or their zymogens which can, under favorable conditions, accelerate the chemical disintegration of tissue proteins. The pathologists also have attempted to associate various abnormal reactions with the existence of autolytic processes, tending to emphasize the biological importance of such an arrangement.<sup>3</sup>

Admitting the probable significance of autolytic functions in living tissues, one might naturally expect to find variations in the occurrence of the appropriate enzymes, associated with changes in the nutritive condition of the individual and the specific organs

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> For discussion of some of these views and the older literature on this subject, see LEVENE: Autolysis, The Harvey lectures, 1905-1906; JACOBY: *Ergebnisse der Physiologie*, 1902, i, I, p. 213.

<sup>3</sup> Cf. OSWALD: *Biochemisches Centralblatt*, 1904-1905, iii, p. 365.

examined. Studies in this direction have been made, among others, by Schlesinger.<sup>4</sup> The liver was employed as the representative organ for examination, the intensity of autolytic activity being measured by the increase in non-coagulable nitrogenous compounds. Schlesinger believed that in disease involving digestive disturbances there occurs a diminished autolytic power. In atrophic conditions, especially in infants, the decrease was reported as particularly noteworthy. The intensity of autolysis was considered as maximal in new-born animals (rabbits), diminishing with increasing age. Evidence obtained from human embryos which had died *in utero* indicated that autolytic reactions can take place under intra-uterine conditions.

More recently, however, knowledge has been obtained of factors which make uncertain any unqualified conclusions respecting the quantitative relations involved in autolysis of tissues. The existence of substances antagonistic to the activity of tissue enzymes has been discovered, and the influence of the reaction of the digesting medium has been recognized more clearly than heretofore. Wiener<sup>5</sup> has shown that autolytic enzymes scarcely act until the tissues become acid, alkaline reaction exerting a distinct retarding influence. Similar results have been demonstrated by Schryver.<sup>6</sup> Von Drjewecki<sup>7</sup> has found that autolysis will proceed in alkaline solution, though at a very much slower rate than in acid media. Obviously experiments in which the comparative reaction of the digesting mixtures is not taken into consideration cannot give conclusive data respecting the relative autolytic powers of the tissues investigated. This criticism may be applied to the work of Schlesinger previously quoted.<sup>8</sup>

In continuation of our studies on the chemico-physiological equipment of growing tissues we have attempted to ascertain to what extent embryonic organs are supplied with autolytic powers. Since in the adult the liver is one of the organs which shows the greatest tendency toward rapid autolysis after removal from the body, our experiments were confined to this gland. The accelerating influence of acids was speedily recognized in our work as a factor of primary

<sup>4</sup> SCHLESINGER: Beiträge zur chemischen Physiologie, 1904, iv, p. 87. This paper reviews much of the earlier literature on autolysis.

<sup>5</sup> WIENER: Zentralblatt für Physiologie, 1905, xix, p. 349.

<sup>6</sup> SCHRYVER: Bio-chemical journal, 1906, i, p. 149.

<sup>7</sup> VON DRJEWECKI: Biochemische Zeitschrift, 1906, i, p. 228; cf. also PRETI: Zeitschrift für physiologische Chemie, 1907, lii, p. 485.

<sup>8</sup> SCHLESINGER states, p. 96, that "Die Reaktion wird ganz schwach sauer."

importance in comparing data derived from different tissues. Several investigators have studied the *rate* of autolytic change in tissue extracts with a view to deciding whether the progress of the reaction is influenced in any specific way under different nutritive conditions.<sup>9</sup> Since the factors possibly involved are too diverse and uncertain at present to admit of a conclusive control and interpretation of the *rate* of autolysis in its early stages, we have determined the extent of autolysis at the end of sixteen hours and sometimes forty-two hours also. The increase in formation of non-coagulable nitrogenous disintegration products after sixteen hours is very slight, corresponding with the experience of previous investigators who have studied the rate of self-digestion of liver.<sup>10</sup> Having convinced ourselves of this, we usually stopped the experiments at the end of sixteen hours; and our comparisons are in general based upon results thus obtained. The tissues used were from pigs and pig embryos.

**Method.** — The extent of autolysis was estimated by a comparison of the amount of non-coagulable nitrogenous products formed with that present in a control experiment in which self-digestion was prevented. Three grams of the finely pulped liver were accurately weighed out and 30 c.c. of water and 2 c.c. of toluene added. The mixtures were allowed to stand at 38° in a stoppered bottle, being shaken one to three times at intervals during sixteen hours. In control experiments the mixtures were immersed in a boiling water bath during fifteen minutes to stop enzymatic action and then subjected to the same treatment as the autolyzing mixtures. To determine the extent of proteolytic digestion the analytical procedure suggested by Schryver<sup>11</sup> was adopted with a few minor modifications. The mixture was treated with an equal weight (roughly) of anhydrous sodium sulphate in a round-bottomed glazed dish.<sup>12</sup> After standing three or four days the hardened mass was powdered in a mortar, transferred to a Jena glass flask and boiled about three quarters of an hour with absolute alcohol with a reflux condenser.

<sup>9</sup> Cf. LANE-CLAYTON and SCHRYVER: *Journal of physiology*, 1904, xxxi, p. 175; SCHRYVER, *Ibid.*, 1905, xxxii, p. 159; WELLS and BENSON: *Journal of biological chemistry*, 1907, iii, p. 35.

<sup>10</sup> Cf. LANE-CLAYTON and SCHRYVER: *Loc. cit.*; also WELLS and BENSON: *Loc. cit.*

<sup>11</sup> SCHRYVER: *Bio-chemical journal*, 1906, i, p. 131.

<sup>12</sup> This is preferable to the flat-bottomed dish recommended by SCHRYVER. If the cake is inverted just after it has commenced to harden, the subsequent sticking of the material to the dish can be avoided.

## SUMMARY OF THE EXPERIMENTS.

ADULT LIVERS.						
	Duration of autolysis.	Total N.	Coagulum N.	Acidity. $n/10$ NaOH.	Extent of autolysis. Total N.	
	hrs.	c.c.	c.c.	c.c.	per cent	
1	0	34.9	28.7	..	..	
	16		22.0	..	19	
	42		20.3	..	24	
2	0	33.4	26.7	..	..	
	16		17.0	..	29	
	42		16.7	..	30	
3	0	34.8	28.3	..	..	
	16		17.1	..	32	
	42		16.3	..	34	
4	0	33.1	27.8	..	..	
	16		16.5	..	34	
	42		17.9	..	30	
5	0	29.9	24.1	..	..	
	16		12.5	..	39	
	42		13.4	..	37	
6	0	29.3	22.0	..	..	
	16		17.2	2.3	16	
	16 + 1 c.c. $n/10$ acid		14.3	3.7	26	
7	0	33.3	26.3	..	..	
	16		21.1	..	16	
	(a) 16 + 1 c.c. $n/10$ acid		17.5	..	26	
8 <sup>1</sup>	(b) "	25.3 <sup>1</sup>	18.0	..	25	
	0		21.3	..	..	
	16		13.0	..	33	
9	16 + 0.8 c.c. $n/10$ acid	32.8	11.3	2.8	39	
	0		26.9	..	..	
	16		17.0	4.4	30	
	16 + 1 c.c. $n/10$ acid		17.0	..	30	
<sup>1</sup> Only 2.5 gm. of liver tissue were used in this experiment.						
EMBRYO LIVERS.						
	Size of embryos.	Duration of autolysis.	Total N.	Coagulum N.	Acidity. $n/10$ NaOH.	Extent of autolysis. Total N.
	mm.	hrs.	c.c.	c.c.	c.c.	per cent
10	50	0	25.4	20.5	..	..
		16		17.3	..	12
		42		17.4	..	12
11	50	0	26.8	22.4	..	..
		16		18.4	..	15
		42		18.8	..	13
12	62	0	24.3	20.9	..	..
		16		17.9	..	12
		16 + 1 c.c. $n/10$ acid		12.9	..	33
13	75	0	26.7	20.7	..	..
		16		19.3	..	5
		42		19.1	..	6



## SUMMARY OF THE EXPERIMENTS (continued).

	Size of embryos.	Duration of autolysis.	Total N.	Coagulum N.	Acidity. $n/10$ NaOH.	Extent of autolysis. Total N.
	mm.	hrs.	c.c.	c.c.	c.c.	per cent
14	75	0	26.7	20.4	..	..
		16		17.9	..	9
		42		17.7	..	10
15	100	0	25.5	20.8	..	..
		16		19.5	..	5
		42		19.9	..	4
16	100	0	26.2	20.6	..	..
		16		18.8	..	7
		42		18.5	..	8
17	100	0	25.0	19.4	..	..
		16		18.2	0.7	5
		16 + 1 c.c. $n/10$ acid		12.2	2.8	29
18	120	0	25.8	19.6	..	..
		16		18.4	..	5
		42		17.8	..	7
19	120	0	25.6	20.8	..	..
		16		18.2	1.3	10
		42		18.0	..	11
20	120	0	24.4	20.6	..	..
		16		18.5	..	9
		16 + 1 c.c. $n/10$ acid		14.3	..	26
21	150	0	26.4	21.5	..	..
		16		18.9	..	10
		42		18.3	..	12
22	150	0	25.8	20.4	..	..
		16		18.1	..	9
		42		16.8	..	14
23	150	0	24.5	20.1	..	..
		16		18.9	0.8	5
		16 + 1 c.c. $n/10$ acid		13.5	2.0	27
24	175	0	25.9	20.6	..	..
		16		18.8	..	7
		42		18.8	..	7
25	175	0	22.4	18.0	..	..
		16		14.7	..	15
		16 + 1 c.c. $n/10$ acid		13.0	..	22
26	200	0	23.5	18.3	..	..
		16		17.5	..	3
		42		17.0	..	6
27	200	0	24.5	20.5	..	..
		16		17.5	..	12
		42		17.5	..	12
28	200	0	23.1	17.9	..	..
		16		16.0	0.9	8
		16 + 1 c.c. $n/10$ acid		10.6	3.1	32
29	215	0	21.5	16.5	..	..
		16		13.5	1.5	14
		42		13.5	..	14
30	250	0	22.2	18.2	..	..
		16		14.2	1.9	18
		16 + 1 c.c. $n/10$ acid		11.3	2.7	31
31	280	0	21.2	17.8	..	..
		16		15.1	..	12
		42		14.4	..	19

The alcohol was decanted through papers on a Buchner funnel, the residue in the flask being heated on a steam bath with water to render coagulable proteins insoluble and dissolve the non-coagulable nitrogenous compounds and the sodium sulphate. The coagulum was filtered through the same papers,<sup>12</sup> and the nitrogen of the washed coagulum estimated by the Kjeldahl-Gunning process. The total nitrogen content of the fresh tissue was also determined. All the results are expressed in cubic centimetres of  $n/5$  acid used to neutralize the ammonia formed in the Kjeldahl estimation. The proportion of the total nitrogen rendered soluble and non-coagulable during autolysis is also calculated from these data. The acidity of the digestion mixtures was determined by titration with  $n/10$  NaOH using litmus as an indicator. The figures given represent the volumes of the alkali solution used. Where the mixtures were acidified before autolysis,  $n/10$  acetic acid was employed.

**Discussion of the results.** — A survey of the data summarized in the tables indicates that the proportion of N in the form of soluble (non-coagulable) constituents in the fresh livers of both the adult and embryo pig is fairly constant, amounting to about 20 per cent of the total N. That the progress of autolysis is ordinarily practically stationary at the end of sixteen hours under the conditions of our experiments is indicated in the numerous trials where a digestion period of forty-two hours failed to afford more than a very slight increase in soluble N. (*Vide* Experiments 1, 2, 3, 5, 14, 15, 16, 21, etc.) In a large number of control experiments in which boiled tissue mixtures were allowed to stand side by side with the autolyzing products and examined under comparable conditions, the analyses gave figures practically duplicating those obtained for the fresh tissues. The brief stay in the thermostat is therefore insufficient to induce any hydrolysis or solvent action on the proteins independent of the tissue enzymes. The data for these control trials are not included in the tables above. A few typical protocols selected at random illustrate the statement just made (see next page).

In the adult livers the extent of autolysis, expressed in percentage ratio of soluble N to total N, tended to approach one third of the total nitrogen present. (*Vide* Experiments 2, 3, 4, 5, 8, 9.) The accelerating influence of acids is distinctly shown (*cf.* Experiments 6, 8), except in trials (*cf.* Experiment 9) where the acidity developed in the autolyzing mixture was itself adequate to afford

<sup>12</sup> This filtration was very slow, especially with the fresh (undigested) tissue.

maximal results. The normal variations in the rate of autolysis noted above may be attributed to normal variations in the quantities of facilitating acids liberated in the tissue or to varying proportions of blood left in the organ. Blood serum exerts a strongly inhibitory action on autolysis.<sup>14</sup> The unlike production of organic acids which favor the autolytic changes<sup>15</sup> may be associated with the

## COAGULUM-N IN PREVIOUSLY HEATED MIXTURES.

(c.c.  $n/5$  acid used.)

No. of Experiment	1	2	9	7	8	16	14	19	23	25	24	30
Fresh tissue . .	28.7	26.7	26.9	26.3	21.3	20.6	20.4	20.8	20.1	18.0	20.6	18.2
After 16 hrs. . .	29.0	28.5	26.8	28.2	21.1	20.9	21.6	20.1	20.4	18.6	21.1	17.7
After 42 hrs. . .	28.9	28.1	..	..	..	20.5	21.5	20.5	..	..	20.5	..

unlike nutritive condition of the animals which furnished the livers. Magnus-Levy<sup>16</sup> has demonstrated that in autolysis of the liver lactic acid in particular is formed at the expense of the carbohydrates present. Variations in the glycogen content of the liver might therefore account for the unlike acidity and resulting differences in the extent of autolysis observed.

In the case of the embryo pig the striking result is the slight extent to which autolysis took place under normal conditions in liver mixtures from embryos of all sizes. That this is not due to any specific lack of enzyme in the embryonic tissues is made clear by the experiments in which small amounts of acetic acid were added to the autolysis mixtures. The extent of autolysis was thereby at once increased to a degree comparable with what was noted with the adult livers. (*Vide* Experiments 12 (33 per cent), 17 (29 per cent), 20 (26 per cent), 23 (27 per cent), 28 (32 per cent), 30 (31 per cent).)

In seeking for an explanation of the relatively slight development of acid and consequent failure to obtain pronounced autolysis with the embryonic livers we recall our observations on the absence of

<sup>14</sup> Cf. SCHRIVVER: Bio-chemical journal, 1906, i, p. 144; BAER and LOEB: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liii, p. 1.

<sup>15</sup> Cf. SCHRIVVER: *Loc. cit.*, p. 153; HEDIN and ROWLAND: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 536; ARINKIN: *Ibid.*, 1907, liii, p. 192.

<sup>16</sup> MAGNUS-LEVY: Beiträge zur chemischen Physiologie, 1902, ii, p. 283.

carbohydrates from these tissues.<sup>17</sup> In the pig the embryo liver is devoid of glycogen, — one of the sources of the acidity developed in autolysis, according to the experiments of Magnus-Levy to which reference was made above. It is interesting to note that the extent of autolysis in our trials without addition of acid increases with the acidity developed in the reaction. Thus

In Experiment 17, acidity = 0.7 c.c., autolysis 5 per cent						
"	"	23	"	0.8	"	5 " "
"	"	28	"	0.9	"	8 " "
"	"	19	"	1.3	"	10 " "
"	"	29	"	1.5	"	14 " "
"	"	30	"	1.9	"	18 " "

The degrees of native acidity here represented are decidedly smaller than in the experiments with adult livers where marked autolysis was obtained. Any specific influences attributable to the ages of the embryos cannot be pointed out. Considering the extent of autolytic change (22–33 per cent) in those experiments (12, 17, 20, 23, 25, 28, 30) in which more favorable conditions were introduced, there is no reason to assume any noteworthy lack of autolytic tissue enzymes in the embryo liver.<sup>18</sup> With the contention of certain investigators that these enzymes play no part in metabolism during life we are not at present concerned. At any rate the equipment for autolytic tissue disintegration is present early in the embryo, even if it is held in check by the normal environment and reaction of the animal cells.

<sup>17</sup> MENDEL and LEAVENWORTH: This journal, 1907, xx, p. 123.

<sup>18</sup> According to VERNON: Journal of physiology, 1905, xxxiii, p. 99, the *erepsin* content of tissues is related to their functional capacity. The *ereptic* power is said to increase considerably during intra-uterine development and for the first few days of post-natal existence, but remains constant during subsequent growth.

## CHEMICAL STUDIES ON GROWTH.—VI. CHANGES IN THE PURINE-, PENTOSE-, AND CHOLESTEROL- CONTENT OF THE DEVELOPING EGG.<sup>1</sup>

BY LAFAYETTE B. MENDEL AND CHARLES S. LEAVENWORTH.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

### THE SYNTHESIS OF PURINES IN THE EMBRYO.

AT the present time there is no convincing experimental evidence of the synthetic formation of purine complexes in the adult mammalian organism.<sup>2</sup> The synthetic production of uric acid as an end-product of nitrogenous metabolism in birds and reptiles is a distinctive reaction associated with excretory functions in these species, without a demonstrated parallel in mammals. Since nucleic acid complexes form an integral part of the chemical components of typical cells everywhere, one would expect the constituent purine bases to be built up in growing tissues. The question of purine synthesis in developing organisms has received little attention from investigators. Tichomiroff<sup>3</sup> contributed the first data bearing on this question. He analyzed the eggs of insects (*Bombyx mori*) for purine bases by the older methods of Kossel and also made similar estimations when the larval forms were developed after an incubation period of thirteen days at 23° C. The purine bases showed an increase from 0.02 gm. per 100 gm. of eggs to 0.2 gm. at the end of the period indicated, giving distinct evidence of a synthetic process. The nature of the individual bases present was determined, but need not be considered here in

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> The literature on this question is reviewed by WIENER: *Ergebnisse der Physiologie*, 1902, i, I, p. 613; BURIAN: *Medizinische Klinik*, 1905, No. 6, and *Zeitschrift für physiologische Chemie*, 1905, xliii, p. 530; MENDEL: The Harvey lectures, 1905-1906, p. 208, and *Journal of the American Medical Association*, March 31, 1906; PFEIFFER: *Beiträge zur chemischen Physiologie*, 1907, x, p. 324.

<sup>3</sup> TICHOMIROFF: *Zeitschrift für physiologische Chemie*, 1885, ix, p. 518.

view of the presumable inadequacy of the older analytical methods employed. Kossel<sup>4</sup> made a similar study of the purine content of the hen's egg before and after incubation. He failed to detect purine bases in the unchanged egg, whereas the embryos separated after fifteen 'days' development yielded 0.28 per cent of guanine and 0.66 per cent of hypoxanthine (and adenine?). These data also were obtained at a time when the present methods of isolation of the individual purines were not yet perfected, and must therefore be considered as indicative merely of a synthetic process without demonstrating conclusively the nature of the bases present. Such formative processes are unquestionably associated with the elaboration of nuclear materials.

The possibility of synthetic production of purine bases in the growing mammal was demonstrated by Burian and Schur<sup>5</sup> for suckling animals. In various litters individuals were analyzed at birth, and their content of purine bases compared with that of the remaining animals which were allowed to grow on a diet consisting exclusively of milk, a food notably poor in purines. The evidence was conclusive that purine compounds can be synthesized from other complexes in the proteins. The nature of these sources has remained a matter of speculation.<sup>6</sup>

We have collected further experimental data on the synthesis of purines in developing eggs. The analytical results obtained in such an investigation are particularly suggestive because one begins with materials practically free from purines and no contribution is made from without during the developmental period. The sources of the synthetic product are presumably the proteins of the egg. Our attempts were in part directed toward ascertaining by competent methods whether the bases thus formed are the same as those found in the tissues of the adult.

#### TOTAL PURINE CONTENT OF EGGS AT VARIOUS STAGES OF DEVELOPMENT.

**Method.** — The entire contents of the shell were extracted with hot alcohol and the residues heated with 5 per cent sulphuric acid

<sup>4</sup> KOSSEL: *Zeitschrift für physiologische Chemie*, 1886, x, p. 248.

<sup>5</sup> BURIAN and SCHUR: *Zeitschrift für physiologische Chemie*, 1897, xxiii, p. 55.

<sup>6</sup> Compare, for example, KOSSEL: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 349; BURIAN: *Ergebnisse der Physiologie*, 1906, v, p. 821.

at 100° during sixteen hours. The material was then filtered and the residue again heated eight hours with fresh acid. The united

## PURINE CONTENT.

	No. of eggs used.	Period of Incubation.	Total Purine N.	Purine N per egg.	Method.
EXPERIMENTS WITH HENS' EGGS.					
a	12	days. 0	gm. 0.019	gm. 0.0016	Double precipitation with copper- sulphate-bisulphite reagents.
b	4	7	0.0267	0.0066	
c	4	14	0.046	0.0115	
d	4	21	0.068	0.017	
e	4	21	0.088	0.022	
f <sup>1</sup>	4	11	0.022	0.0055	Additional precipitation with sil- ver reagent.
g	4	14	0.049	0.012	
h	4	21	0.093	0.023	
i <sup>1</sup>	4	21 + 14 feeding	0.098	0.0246	Additional precipitation with sil- ver reagent.
j <sup>1</sup>	4	21 + 14 feeding	0.101	0.0253	
k <sup>1</sup>	4	21 + 14 feeding	0.100	0.025	
EXPERIMENTS WITH DUCKS' EGGS.					
l	2	9	0.0056	0.0028	Double precipitation with copper- sulphate-bisulphite reagents.
m	2	18	0.044	0.022	
n	2	27	0.059	0.030	
<sup>1</sup> In these experiments the contents of the egg-shell were not extracted with hot alcohol.					

filtrates were neutralized with sodium hydrate, concentrated<sup>7</sup> and filtered. The quantitative estimation of the purines was made in the resulting solutions by the Krüger-Schmid method,<sup>8</sup> a double

<sup>7</sup> This effected the separation (in the saturated Na<sub>2</sub>SO<sub>4</sub> solution) of proteose-like material which interferes with the subsequent purine precipitations.

<sup>8</sup> KRÜGER and SCHMID: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 1.



precipitation with the copper-bisulphite reagents always being carried out. In some series this was followed by a silver precipitation. The progressive increase in purine nitrogen during the period of incubation is uniformly found, giving evidence of the synthesis of the bases as a characteristic feature of development. The question may at once be raised whether we are not dealing here with uric acid, formed by the same agencies which give rise to it synthetically in adult birds, rather than with a production of purine groups in the form of nucleic acid. That this is not the case is shown by the further analyses reported below in which the purine bases were isolated and shown to be those characteristic as components of nucleoproteins.

In Experiments i, j, k, the chicks, after hatching, were fed two weeks exclusively upon bread crumbs containing practically no purines (0.008 per cent purine N). The purine content of the body was not noticeably increased during this period.

#### SEPARATION OF THE PURINES.

**Method.** — The entire contents of the egg-shells were extracted with hot alcohol and ether and the residues decomposed by heating with 5 per cent sulphuric acid until the solutions no longer gave the biuret reaction. In one experiment the purine bases were first precipitated with Hopkins' mercuric sulphate solution,<sup>9</sup> and then, after removal of the mercury with hydrogen sulphide, by means of the copper-sulphate-bisulphite reagents. In the other experiments the Krüger-Schmid process alone was employed. The separations and identification of the bases proceeded as described in an earlier communication.<sup>10</sup>

These figures bring further evidence of the progressive synthesis of purines taking place during the growth of the embryo, and they show that, as in the adult and embryo organs already examined,<sup>11</sup> guanine and adenine are the predominating bases involved.

<sup>9</sup> Cf. LEVENE: This journal, 1904-1905, xii, p. 276.

<sup>10</sup> This journal, 1907, xx, p. 102.

<sup>11</sup> Cf. This journal, 1907, xx, p. 103.

## PURINE BASES.

Number of eggs used.		Period of Incubation.	PURINE BASES ESTIMATED.										
			Total.			Per egg.							
			Guan-ine.	Aden-ine.	Hypoxan-thine.	Guan-ine.	Aden-ine.	Hypoxan-thine.					
HENS' EGGS.													
o <sup>1</sup>	57	days. 21 <sup>2</sup>	gm. 0.294 <sup>3</sup>	gm. 0.183 <sup>3</sup>	gm. 0.018	gm. 0.005	gm. 0.003	gm. 0.0003					
p	53	20	0.279	0.187 <sup>7</sup>	0.015	0.005	0.0035	0.0003					
q	212	{ embryos } { 1164 gm. } { yolks } { 8370 gm. } { total egg } { content }	{ 0.203 <sup>4</sup>	0.198 <sup>8</sup>	. . .	0.0009	0.0009						
	{ 0.117 <sup>4</sup>								0.108 <sup>9</sup>	. . .	0.0006	0.0005	
	{ 0.320 <sup>4</sup>												
DUCKS' EGGS.													
r	21	28 <sup>2</sup>	0.211 <sup>5</sup>	0.232 <sup>10</sup>	0.040	0.010	0.011	0.0002					
<div><div><div><div><div><sup>1</sup> In this experiment the purines were first precipitated with the Hopkins reagent.</div><div><sup>2</sup> Just hatched.</div><div><sup>3</sup> 0.4354 gm. C<sub>5</sub>H<sub>5</sub>N<sub>5</sub>O · HCl · 2 H<sub>2</sub>O.</div><div><sup>4</sup> 0.474 gm. " "</div><div><sup>5</sup> 0.312 gm. " "</div><div><sup>6</sup> 0.5195 gm. C<sub>5</sub>H<sub>5</sub>N<sub>5</sub> · C<sub>6</sub>H<sub>2</sub>(NO<sub>2</sub>)<sub>3</sub>OH · H<sub>2</sub>O.</div><div><sup>7</sup> 0.529 gm. " " "</div><div><sup>8</sup> 0.5615 gm. " " "</div><div><sup>9</sup> 0.3066 gm. " " "</div><div><sup>10</sup> 0.656 gm. " " "</div></div><div><div>N = 31.15 %; calculated 31.3 %.</div><div>H<sub>2</sub>O = 16.1 %; calculated 16.1 %.</div><div>N = 37.2 % in the dehydrated salt.</div><div>H<sub>2</sub>O = 15.6 %.</div><div>N = 37.0 % in the dehydrated salt.</div><div>m.p. 278-80° C.</div><div>m.p. 281°.</div><div>m.p. 282°.</div><div>m.p. 282°.</div><div>m.p. 279°.</div></div></div></div></div>													

## THE PENTOSE CONTENT OF DEVELOPING EGGS.

The tissue nucleoproteins are usually considered to contain constituent pentose groups,<sup>12</sup> since they yield furfural on distillation

<sup>12</sup> A recent discussion of this point will be found by v. FÜRTH and JERUSALEM: *Beiträge zur chemischen Physiologie*, 1907, x, p. 180.

with hydrochloric acid. Inasmuch as the purines enter into the make-up of the nucleoprotein complexes, it became of interest to learn whether pentose groups exist preformed in the egg or are formed *pari passu* with the purines. That the pentose content of a tissue is proportional to its richness in nuclei is suggested by a research of Neuberg.<sup>13</sup> Experiments on the liver of the embryo pig have convinced us of the general probability of this relation. We have repeatedly demonstrated that the fresh eggs of the hen and duck fail to yield furfural on decomposition. A summary of analyses of the egg contents at various periods of incubation is given below. The materials were dried with the aid of alcohol and heat, weighed, and then ground as finely as possible. Three to five grams were treated by Tollens' method<sup>14</sup> and the furfural phloroglucid weighed. The experiments were repeated with numerous eggs, duplicate analyses being made in each case. The results are expressed in equivalents of the phloroglucid per egg.

## SUMMARY.

	Period of Incubation.	Furfural phloroglucid per egg.
	days	gm.
Hens' eggs . . . . {	7	none
	14	0.012
	21	0.020
	21	0.024
Ducks' eggs . . . . {	9	none
	18	trace
	27	0.037

## THE FAT AND CHOLESTEROL CONTENT OF DEVELOPING HENS' EGGS.

The more familiar chemical changes in developing birds' eggs, such as the variations in their content of water and proximate principles, have been subject to investigation since many years.<sup>15</sup> They

<sup>13</sup> NEUBERG: Berliner klinische Wochenschrift, 1905, p. 118. Cf. also BEEBE and SHAFFER: This journal, 1905, xiv, p. 231.

<sup>14</sup> TOLLENS: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 239.

<sup>15</sup> Cf. POTT: Landwirtschaftliche Versuchs-Stationen, 1878, xxiii, p. 203; PREYER: Specielle Physiologie des Embryo, 1885, p. 274; and LIEBERMANN: Archiv für die gesammte Physiologie, 1888, xliii, p. 71, where the references to earlier literature will be found.

show a decrease in the total quantity of ether-soluble substances present within the shell. Parke<sup>16</sup> long ago found that both the ether extract and the alcohol extract of the hen's egg-yolk diminishes in quantity during the period of incubation. The cholesterol changes similarly. Parke's figures for the yolk are:

	Ether Extract.	Cholesterol.
Fresh egg . . . . .	4.453 gm.	0.248 gm.
10 days' incubation . . .	4.280	0.230
17 days' incubation . . .	3.961	0.163

There is simultaneously an increase in the fat content of the enlarging embryo itself; but according to the figures on record this is insufficient to account for the gradual loss of fatty materials from the remaining yolk and albumen, especially in the later stages of incubation. It is likely, therefore, that fat contributes to the expenditure of energy, which is generally recognized to occur during embryonic life. The gaseous metabolism at this period may apparently be very vigorous.<sup>17</sup> The most conspicuous changes occur toward the end of the embryonic period.

We have incidentally endeavored to learn whether cholesterol likewise shares in these changes—whether any alteration occurs in the total stock of cholesterol available in the entire egg. Parke's observations on the yolk have been recorded above.

In the eggs of the insect *Bombyx mori*, Tichomiroff<sup>18</sup> found slight changes in the ether extract from 9.52 to 6.46 per 100 gm. eggs and 0.40 to 0.35 in the cholesterol during the period of incubation. In our experiment the entire contents of the egg-shell were rubbed with an equal weight of anhydrous sodium sulphate, and the hardened mass ground up and extracted five times with boiling ether. The dried ether extract was weighed and then examined for cholesterol by Ritter's method.<sup>19</sup> The purity of the cholesterol weighed is evidenced by the melting point of each specimen. The data are summarized in the table.

<sup>16</sup> PARKE: Hoppe-Seyler's Medizinisch-chemische Untersuchungen, 1867, p. 211. For lecithin cf. MESERNITZKY: Biochemisches Centralblatt, 1907, vi, p. 784.

<sup>17</sup> Cf. HASSELBACH: Skandinavisches Archiv für Physiologie, 1899-1900, x, p. 353; BOHN: *ibid.*, p. 413.

<sup>18</sup> TICHOMIROFF: Zeitschrift für physiologische Chemie, 1884-1885, ix, p. 531.

<sup>19</sup> RITTER: Zeitschrift für physiologische Chemie, 1901-1902, xxxiv, p. 430.

## SUMMARY.

No. of eggs.	Period of Incubation.	Weight of contents.	Ether Extract.		Cholesterol.	
			Total.	Per egg.	Total.	Per egg.
	days.	gm.	gm.	gm.	gm.	gm.
3	0	55.8				
		51.5 } 155.8	16.5	5.5	1.135 <sup>1</sup>	0.378
		48.5 }				
3	14	45.1				
		44.1 } 129.4	13.8	4.6	0.826 <sup>2</sup>	0.275
		40.1 }				
3	21 (hatched)	37.4				
		34.4 } 106	8.7	2.9	0.751 <sup>3</sup>	0.250
		34.1 }				
<sup>1</sup> m. p. 144-45°.			<sup>2</sup> m. p. 144-45°.		<sup>3</sup> m. p. 145°.	

These data are comparable with the somewhat lower figures obtained by Parke for the egg-yolk many years ago. They give no evidence of a synthesis of cholesterol in the processes incidental to the development of the chick. On the contrary, the cholesterol appears to disappear in part in company with the rest of the lipid yolk substances which act here as sources of energy in growth.

## CHEMICAL STUDIES ON GROWTH.—VII. THE CATALASE OF ANIMAL EMBRYONIC TISSUES.<sup>1</sup>

BY LAFAYETTE B. MENDEL AND CHARLES S. LEAVENWORTH.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

A CAREFUL interpretation of the abundant and confused data regarding the nature of certain oxidative reactions in animal and plant tissues clearly indicates the occurrence of varied enzymatic properties. At the present stage of experimental inquiry three peculiar groups of enzymes may be postulated: (1) true *oxidases*, more or less specific in action; (2) *peroxidases*, requiring the presence of hydrogen peroxide or organic peroxides to fulfil their activity; (3) *catalases*, capable of liberating oxygen from peroxide.<sup>2</sup> The preponderance of evidence at the present moment suggests that each type of reaction here represented may be independently instituted or accelerated by individual enzymes, although all writers are not yet agreed upon the individuality of the accelerating or activating agent.

In continuation of the study of the chemical equipment of the embryo at various stages of development we have turned our attention to the catalytic decomposition of hydrogen peroxide. This reaction especially has been observed with so great a diversity of tissues that it has in the past often been regarded as a characteristic property of enzymes in general. Since the investigations of Loew, however, the enzyme catalase has received independent recognition.<sup>3</sup>

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> The literature on this subject will be found in papers by BACH and CHODAT: *Biochemisches Centralblatt*, 1903, i, pp. 417, 457; VON CZYHLARZ and VON FÜRTH: *Beiträge zur chemischen Physiologie*, 1907, x, p. 358.

<sup>3</sup> Cf. SHAFFER: *This journal*, 1905, xiv, p. 299; EULER: *Beiträge zur chemischen Physiologie*, 1906, vii, p. 1; cf. also KASTLE and LOEVENHART: *American chemical journal*, 1903, xxix, p. 581.

We favor the view, furthermore, that it is not an oxidizing enzyme in the strict sense of the word and must be distinguished fundamentally from the so-called oxidases.<sup>4</sup> Thus catalase is not necessary for the production of the well-known guaiacum-blue reaction. The oxygen liberated by catalase appears to be in the molecular rather than a nascent or atomic state.

Too great stress must not be placed on the data regarding the relative distribution of catalases; for the methods of comparison are open to criticism, and the presence of inhibitory factors often masks the inherent powers of the material examined. The blood is well known to be rich in catalase (haemase) which has been carefully investigated. Liebermann found relatively blood-free adipose tissue to be rich in catalase; and Euler has argued for the non-identity of the catalases of different origin.<sup>5</sup> Wolfgang Ostwald<sup>6</sup> has noted that sperm is richer in catalase than ovarian substance and has discussed the significance of the enzyme (p. 462). In numerous papers Battelli and Stern<sup>7</sup> have described the distribution of animal catalases in organs and species. In general the relative order of the tissues in content of the enzyme is: liver, kidney, blood, lung, muscle, brain. The significance of outside factors was clearly emphasized by Loevenhart,<sup>8</sup> who has shown how greatly they may modify the velocity of the reaction. Thus acid markedly inhibits the action of catalase, and in many cases the so-called activating action of various substances is due merely to their power to neutralize or bind the acid contained in commercial peroxide. Likewise McGuigan<sup>9</sup> has lately observed that "equal quantities of the various tissues of the body, dried and powdered, require different quantities of acid to slow the catalysis of hydrogen peroxide to a certain point. The kidney requires most acid, then the liver, the spleen, pancreas, and muscle, following in the order named, requiring progressively less

<sup>4</sup> Cf. LIEBERMANN: *Archiv für die gesammte Physiologie*, 1904, civ, p. 203; 1905, cviii, p. 489; VAN LAER: *Centralblatt für Bakteriologie*, ii, 1906, xvii, Heft 17-18; SHAFFER, *loc. cit.*; VON CZYHLARZ and VON FÜRTH: *Loc. cit.*

<sup>5</sup> So LESSER: *Zeitschrift für Biologie*, 1906, xlviii, p. 1; xlix, p. 575, who assumes that catalase liberates *active* oxygen, suggests that the enzymes may be relatively more abundant in the blood of the more actively oxidizing species.

<sup>6</sup> OSTWALD: *Biochemische Zeitschrift*, 1907, vi, p. 409.

<sup>7</sup> See the *Comptes rendus de la société de biologie*, 1904, *et seq.*; *Comptes rendus de l'academie des sciences*, 1904, cxxxviii, p. 923; *Archivio di fisiologia*, 1905, ii, p. 471.

<sup>8</sup> LOEVENHART: *This journal*, 1905, xiii, p. 171.

<sup>9</sup> MCGUIGAN: *This journal*, 1907, xix, p. 197.



acid." Differences in oxygen liberation are thus in some cases due solely to differences in the environment of the catalase, the enzymes probably being identical.

Regarding the physiological significance of the catalases nothing convincing can be stated. They are not to be considered as oxidative enzymes, and it has been suggested that their function consists in regulating the oxidative processes by decomposing the peroxides which tend to develop in animal and plant tissues. Kastle and Loevenhart<sup>10</sup> conclude, on the other hand, that "the power possessed by catalase to decompose hydrogen peroxide is to be looked upon, not as its physiological function, but rather as a property possessed in common by a great many substances capable of combining with oxygen, — a property, by the way, which may never be called into play physiologically."

With regard to the distribution of catalase in embryonic animal tissues Battelli and Stern<sup>11</sup> state that they contain less catalase than those of the adult. Buxton and Shaffer<sup>12</sup> note that catalase is always found even in very young embryonic tissue, but the reactions with embryos are weaker than with normal tissues. Herlitzka<sup>13</sup> has likewise reported the occurrence of catalase in the earliest stages of embryonic development.

**Method of experiment.** — The catalytic decomposition of the hydrogen peroxide was studied by the method employed by Loevenhart.<sup>14</sup> The tissues were obtained fresh from the slaughter house and were used within two hours after the death of the animal. Euler<sup>15</sup> has found that fresh catalase-containing extracts retain their activity almost unchanged at least twenty-four hours, but are greatly weakened by acid. We have conducted experiments on the tissues of both full-grown and embryo pigs, using commercial "Oakland Dioxygen" both with its usual acid reaction and also after neutralization. Readings of the volume of oxygen liberated from the peroxide solution at room temperature were taken at intervals of fifteen seconds. The content of the tissue extracts in solid matter was also determined for comparison.

**Results and conclusions.** — Only a very few of the numerous trials

<sup>10</sup> KASTLE and LOEVENHART: *American chemical journal*, 1903, xxix, p. 586.

<sup>11</sup> BATTELLI and STERN: *Archivio di fisiologia*, 1905, ii, p. 471.

<sup>12</sup> BUXTON and SHAFFER: *Journal of medical research*, 1905, viii, p. 549.

<sup>13</sup> HERLITZKA: *Biochemisches Centralblatt*, 1907, vi, p. 234.

<sup>14</sup> LOEVENHART: *This journal*, 1905, xiii, p. 171.

<sup>15</sup> EULER: *Beiträge zur chemischen Physiologie*, 1906, vii, p. 4.

are reported below as illustrative protocols. The experiments clearly indicate the inhibitory influence of the acid in commercial hydrogen peroxide solutions, the velocity of the oxygen liberation being greatly increased in the neutral solutions, particularly with certain tissues. The embryonic tissue extracts appear to be relatively more sensitive to the acid reaction than those from the full-grown animals (contrast Experiments 1, 2, 3 with 7, 11, 15). Like Battelli and Stern, we find adult liver and kidney standing first in order of richness in catalase, the lung, muscle, and nervous tissue being inferior in this respect in about the order named. In the pig embryo these tissues stand in the order: liver, kidney, lung, muscle, and brain. Our protocols fail to show any noteworthy lack of catalase in the youngest embryos within the ranges of 65 to 230 mm., corresponding to fifty to ninety-six days.<sup>16</sup> When the foetal organs are compared with the adult tissues in respect to the volume of oxygen liberated per unit of solid material in the extracts, the latter show a decidedly greater catalytic power with the unneutralized commercial peroxide. With the neutral peroxide, however, these differences largely disappear, and they must accordingly be attributed to differences in the power of the extracts to overcome the inhibitory influence of the acid present rather than in content of catalase. To draw any far-reaching conclusions of functional significance from these facts appears unwarranted so long as the numerous variable factors, such as blood content, possible presence of anti-catalase, and other features of the chemical environment are unknown. It is sufficient here to record the early presence of the catalytic power in embryonic tissues.

<sup>16</sup> Cf. This journal, 1907, xx, p. 90.

## TYPICAL PROTOCOLS.

The figures in the peroxide columns indicate volumes of oxygen liberated in c.c.

## TISSUES OF FULL-GROWN ANIMALS.

EXPERIMENT 1.		Acid peroxide.		Neutral peroxide.	
Part of animal.	Time in seconds.	1 c.c. extract, 4 c.c. water.	2 c.c. extract, 3 c.c. water.	1 c.c. extract, 4 c.c. water.	2 c.c. extract, 3 c.c. water.
Liver	15	14.5	43.3	58.8	62.0
	30	22.3	54.0	60.4	...
	45	32.1	60.4	...	...
	60	40.5	63.9	...	...
	75	49.9	64.3	...	...
	90	54.9	...	...	...
	105	57.6	...	...	...
	120	59.5	...	...	...
Solids in extract used, in mgm.		30	60	30	60
EXPERIMENT 2.		1 c.c. extract, 1 c.c. water.	2 c.c. extract, 3 c.c. water.	1 c.c. extract, 1 c.c. water.	1 c.c. extract, 4 c.c. water.
Kidney	15	8.6	26.0	66.4	67.4
	30	13.4	38.8	67.0	...
	45	21.6	50.7	...	...
	60	28.4	58.7	...	...
	75	36.2	64.6	...	...
	90	42.2	67.0	...	...
	105	47.9	68.0	...	...
	120	52.5	68.9	...	...
Solids in extract used, in mgm.		24	48	24	24
EXPERIMENT 3.		1 c.c. extract, 1 c.c. water.	2 c.c. extract, 3 c.c. water.	1 c.c. extract, 1 c.c. water.	2 c.c. extract, 3 c.c. water.
Lung	15	1.4	2.1	8.3	20.0
	30	2.9	5.5	10.6	26.3
	45	..	7.9	12.0	29.8
	60	..	9.2	...	32.1
	75	4.0	11.8	13.2	34.6
	90	..	12.3	14.6	36.8
	105	5.2	14.4	15.9	39.0
	120	..	15.6	17.0	41.2
Solids in extract used, in mgm.		15	30	15	30

EXPERIMENT 4.			
Part of animal.	Time in seconds.	Acid peroxide. 5 c.c. extract.	Neutral peroxide. 5 c.c. extract.
Muscle <sup>1</sup> . . . . .	15	3.3	..
	30	4.5	1.4
	45	5.8	..
	60	..	..
	75	7.1	2.6
	90	..	..
	105	..	4.4
	120	..	..
EXPERIMENT 5.			
Part of animal.	Time in seconds.	Acid peroxide. 5 c.c. extract.	Neutral peroxide. 5 c.c. extract.
Brain . . . . .	15	5.6	5.6
	30	8.4	9.8
	45	9.6	11.1
	60	10.6	12.5
	75	...	13.4
	90	12.0	...
	105	...	...
	120	...	...
Solids in extract used, in mgm. . . . .		105	105
<sup>1</sup> This experiment was carried out with fresh rabbit's muscle.			



Part of animal.	Time in sec-onds.	EXPERIMENT 10. 65 mm. embryos.						EXPERIMENT 11. 100 mm. embryos.						EXPERIMENT 12. 125 mm. embryos.						EXPERIMENT 13. 230 mm. embryos.					
		Acid peroxide.			Neutral peroxide.			Acid peroxide.			Neutral peroxide.			Acid peroxide.			Neutral peroxide.			Acid peroxide.			Neutral peroxide.		
Kidney . .	15	4 c.c. extract,	3 c.c. water,	2 c.c. extract,	2 c.c. water,	4 c.c. extract,	1 c.c. water,	1 c.c. extract,	4 c.c. water,	2 c.c. extract,	2 c.c. water,	4 c.c. extract,	1 c.c. water,	1 c.c. extract,	4 c.c. water,	2 c.c. extract,	2 c.c. water,	4 c.c. extract,	1 c.c. water,	2 c.c. extract,	3 c.c. water,	1 c.c. extract,	4 c.c. water,	3 c.c. extract,	2 c.c. water,
	30	2.0	5.8	2.6	27.2	33.9	..	1.4	11.6	22.7	11.6	17.2	32.9	1.6	1.5	9.1	31.3	2.5	..	18.0	22.5	18.0	22.5	18.0	22.5
	45	7.3	6.3	5.0	40.6	52.0	..	..	..	..	..	..	..	2.8	4.2	11.1	41.0	3.9	1.6	24.5	30.8	24.5	30.8	24.5	30.8
	60	8.3	7.7	6.3	46.0	60.0	..	2.7	18.4	38.1	18.4	19.7	41.9	4.3	6.8	13.4	46.3	5.1	..	28.0	35.9	28.0	35.9	28.0	35.9
	75	9.8	9.5	7.7	51.0	63.8	1.5	..	20.8	45.2	19.7	20.8	45.2	5.5	11.0	14.5	51.2	6.2	..	30.0	38.0	30.0	38.0	30.0	38.0
	90	10.8	..	9.5	53.5	67.0	..	4.0	21.8	48.6	21.8	21.8	48.6	..	13.3	16.1	58.1	7.6	2.6	31.1	40.4	31.1	40.4	31.1	40.4
	105	12.2	10.4	..	56.8	68.2	..	..	..	..	..	..	..	6.8	14.8	..	61.4	..	..	33.3	43.8	33.3	43.8	33.3	43.8
	120	13.6	..	..	58.9	..	..	5.1	23.0	53.5	23.0	23.0	53.5	..	16.1	17.3	65.0	10.0	4.9	35.4	46.0	35.4	46.0	35.4	46.0
Solids in extract used, { in mgm.		56	42	28	56	56	12	24	24	48	24	33	44	11	33	11	44	22	11	11	22	11	11	22	11

EXPERIMENT 14. 65 mm. embryos.				EXPERIMENT 15. 125 mm. embryos.				EXPERIMENT 16. 230 mm. embryos.					
Part of animal.	Time in seconds.	Acid peroxide.		Neutral peroxide.		Acid peroxide.		Neutral peroxide.		Acid peroxide.		Neutral peroxide.	
		4 c.c. extract, 1 c.c. water.	5 c.c. extract.	4 c.c. extract, 1 c.c. water.	5 c.c. extract.	1 c.c. extract, 4 c.c. water.	2 c.c. extract, 3 c.c. water.	1 c.c. extract, 4 c.c. water.	2 c.c. extract, 3 c.c. water.	1 c.c. extract, 4 c.c. water.	2 c.c. extract, 3 c.c. water.		
Lung	15	..	2.0	6.0	10.0	1.7	1.4	18.0	8.0	..	2.9	6.0	18.6
	30	1.4	4.5	7.3	12.1	..	..	21.9	10.2	..	4.1	8.2	30.2
	45	2.5	...	9.0	13.9	..	2.9	24.3	..	..	5.5	9.6	35.1
	60	4.3	5.6	..	..	..	..	25.5	11.7	..	7.9	11.2	38.7
	75	..	7.0	10.1	14.8	4.2	..	26.6	12.8	1.2	8.3	..	42.2
	90	5.6	..	..	..	..	3.9	27.6	14.2	..	10.5	..	44.1
	105	..	8.1	..	..	..	..	28.7	..	..	11.8	12.3	47.5
	120	..	..	11.3	16.2	..	5.3	30.9	..	..	13.0	..	49.4
Solids in extract used, in mgm.		40	50	40	50	11	22	44	22	12	48	12	48



EXPERIMENT 17. 230 mm. embryos.				EXPERIMENT 18. 65 mm. embryos.			
Part of animal.	Time in seconds.	Acid peroxide. 5 c.c. extract.	Neutral peroxide. 5 c.c. extract.	Part of animal.	Time in seconds.	Acid peroxide. 5 c.c. extract.	Neutral peroxide. 5 c.c. extract.
Muscle . . . . .	15	2.5	1.6	Brain . . . . .	15	1.5	1.6
	30	3.7	..		30	2.7	3.0
	45	5.0	2.8		45	..	..
	60	6.5	..		60	..	4.1
	75	7.5	4.0		75	..	..
	90	..	..		90	..	5.3
	105	..	..		105	4.0	..
	120	8.8	5.7		120	..	..
Solids in extract used, in mgm. . . .		50	50	Solids in extract used, in mgm. . . .		50	50

## CHEMICAL STUDIES ON GROWTH.—VIII. THE OCCURRENCE OF LIPASE IN EMBRYONIC ANIMAL TISSUES.<sup>1</sup>

BY LAFAYETTE B. MENDEL AND CHARLES S. LEAVENWORTH.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE possible rôle of lipases in the metabolism of fat in animal tissues has lately attracted the attention of physiologists, especially since the kinetics of lipolytic reactions have been more carefully investigated and their reversible nature determined.<sup>2</sup> Fat-splitting enzymes are known to be widely distributed in different organs; and the lipolytic power is by no means restricted to the hydrolysis of the true fats, but has been found to exert itself upon a variety of esters of mon-atomic alcohols, phosphatides, and related compounds. Since embryonic organisms are as a rule considered to be comparatively deficient in the enzymes characteristic of the adult forms,<sup>3</sup> studies of the occurrence of unorganized ferments in the embryo have been reported in earlier papers in this series. We desire now to add the results of a study of the ester-splitting power of some embryonic tissues.

Wohlgemuth<sup>4</sup> has demonstrated the existence of lipase in the hen's egg and located the enzyme in the yolk. Since it is currently believed that fats undergo hydrolysis prior to utilization, the dis-

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> For a survey of the general literature on this subject consult KASTLE and LOEVENHART: American chemical journal, 1900, xxiv, p. 491; LOEVENHART: This journal, 1902, vi, p. 331; CONNSTEIN: Ergebnisse der Physiologie, 1904, iii, 1, p. 194; TAYLOR: University of California publications, Pathology, 1907, i, p. 257.

<sup>3</sup> Cf. PREYER: Spezielle Physiologie des Embryo; Leipzig, 1885; KRÜGER, (F): Die Verdauungsfermente beim Embryo und Neugeborenen, Wiesbaden, 1891; VERNON: Journal of physiology, 1905, xxxiii, p. 81; BUXTON and SHAFFER: Journal of medical research, 1905, xiii, p. 549.

<sup>4</sup> WOHLGEMUTH: Zeitschrift für physiologische Chemie, 1905, xlv, p. 540.

covery of lipase in the egg yolk throws light upon the progressive loss of fat reported for the developing egg in an earlier paper.<sup>5</sup> Buxton and Shaffer<sup>6</sup> reported "a trace of lipase" in very small embryos (pig, rabbit, sheep), the enzyme content apparently increasing with the age of the embryos. In the adult animals Kastle and Loevenhart have shown that the hydrolysis of ethyl butyrate is readily accomplished by many tissues, the extracts of liver, gastric, and intestinal mucosa being particularly effective. The reaction is inhibited by acids and many of the common antiseptics used in physiological investigation. Toluene appears to be least objectionable in this respect, and has therefore usually been employed to exclude bacterial action. The enzymes act most favorably at 40° C. Profiting by this experience, we have followed the method of Kastle and Loevenhart,<sup>7</sup> using ethyl butyrate as the zymolyte and 10 per cent "turbid" extracts of the tissues, because they are less sensitive to inhibitory agents. Liver and intestine of the pig were selected as most likely to exhibit the comparative content of lipase. Ten grams of the perfectly fresh tissue were ground with sand, extracted with water, strained through cloth, and the volume made up to 100 c.c. The initial acidity of the extracts — always extremely small — was deducted from the figures obtained for the acidity of the digestion mixtures at the end of each trial. *Blank experiments with boiled tissue extracts uniformly failed to show any increase in acidity.* An illustrative protocol showing the conditions of the experiments follows:

At 39° C. 17 hours	{	0.23 gm. ethyl butyrate <sup>8</sup>
		1 c.c. tissue extract (embryo liver)
		4 c.c. water
		0.1 c.c. toluene
		3.9 c.c. <i>n</i> /20 KOH required to neutralize resulting acidity = 9.8 per cent hydrolysis <sup>8</sup>

Typical results are summarized in the table.

<sup>5</sup> Cf. this journal, 1908, xxi, p. 82.

<sup>6</sup> BUXTON and SHAFFER: *Journal of medical research*, 1905, xiii, p. 549.

<sup>7</sup> KASTLE and LOEVENHART: *American chemical journal*, 1900, xxiv, p. 492; LOEVENHART: This journal, 1902, vi, p. 337; LOEVENHART and others: *Journal of biological chemistry*, 1907, ii (various papers).

<sup>8</sup> 0.23 gm. ethyl butyrate = 0.26 c.c. requires 39.7 c.c. *n*/20 KOH to neutralize the acid which it can yield. The extent of hydrolysis is calculated (after correction for initial acidity) on the basis of these figures. Thus,  $\frac{3.9}{39.7} = 9.8$  per cent.

## THE HYDROLYSIS OF ETHYL BUTYRATE BY TISSUE EXTRACTS.

(Digestions at 39–40° C.)

LIVER.		
Source of the Extract.	Extent of Hydrolysis. (Complete saponification = 100.)	
	After 25 min.	After 17 hours.
50 millimetre embryos <sup>1</sup> . . . .	1.0	5.8
75 millimetre embryos (a) . . .	1.0	4.5
75 millimetre embryos (b) . . .	0.8	5.8
125 millimetre embryos . . . .	0.8	..
150 millimetre embryos . . . .	1.5	4.0
175 millimetre embryos . . . .	1.3	9.8
215 millimetre embryos . . . .	1.0	9.6
full grown pig (a) . . . . .	13.4	30.5
full grown pig (b) . . . . .	14.4	26.7
INTESTINE. <sup>2</sup>		
50 millimetre embryos . . . .	0.5	6.8
75 millimetre embryos (a) . . .	0.8	3.0
75 millimetre embryos (b) . . .	0.8	3.0
125 millimetre embryos . . . .	0.3	1.5
150 millimetre embryos . . . .	0.7	4.0
175 millimetre embryos (a <sub>1</sub> ) . . .	..	4.3
175 millimetre embryos (a <sub>2</sub> ) . . .	..	4.5
215 millimetre embryos (a <sub>1</sub> ) . . .	..	2.3
215 millimetre embryos (a <sub>2</sub> ) . . .	..	2.5
full grown pig (a <sub>1</sub> ) . . . . .	3.3	12.1
full grown pig (a <sub>2</sub> ) . . . . .	3.5	12.3
<sup>1</sup> A compilation indicating the estimated ages of these embryos will be found in MENDEL and MITCHELL: This journal, 1907, xx, p. 90. <sup>2</sup> These experiments were made with 20 per cent extracts of the tissues. The entire intestine was used in the case of the embryos; only the mucosa of the small intestine was employed from the adults.		

No attempt has been made to consider the possible influence of coenzymes or accelerating agents on the embryonic extracts. Nor have we endeavored to determine whether the enzymes concerned in these hydrolyses are identical. Loevenhart<sup>9</sup> believes that the action of at least the liver and pancreas on all of the esters studied

<sup>9</sup> LOEVENHART: Journal of biological chemistry, 1907, ii, p. 427.

is probably attributable to a single enzyme in each of the tissues, although the ester-splitting enzyme in one organ may differ from that in another. An examination of the results presented above gives evidence of the early presence of lipase in the liver and intestine of the embryo; but the activity of the extracts of these tissues is decidedly less pronounced than that of the comparable material from the full-grown animal. This inferiority cannot be ascribed to the lack of epithelial development, since the intestinal lining becomes differentiated early into a specialized cellular structure, in which the sugar-inverting enzymes are easily demonstrated.<sup>10</sup> However, the specific organs early give an intimation of the chemical outfit with which they are endowed more richly at a later period.

<sup>10</sup> Cf. this journal, 1907, xx, p. 81.

CHEMICAL STUDIES ON GROWTH.—IX. NOTES ON  
THE COMPOSITION OF EMBRYONIC MUSCULAR  
AND NERVOUS TISSUES.<sup>1</sup>

By LAFAYETTE B. MENDEL AND CHARLES S. LEAVENWORTH.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

THE chemical data on the composition of developing muscular and nervous structures are exceedingly meagre. We have therefore thought that some of the details ascertained in our embryological studies might have sufficient comparative value to deserve being recorded. The analyses were made upon parts of pig embryos, structures in which Bardeen<sup>2</sup> has shown that the various individual muscles of the trunk become distinct while the embryo is growing from 13 to 30 mm. in length. By the time the embryo has attained a length of 40 to 50 mm. the essential internal architecture of the muscle is apparent. A separation of the muscular and connective tissue components for analytical purposes is, of course, impossible. Neither will analyses of the muscular tissue at this formative period furnish anything more than approximate comparative values, since it is impossible to estimate what proportion of the material used is embryonic connective tissue. Nevertheless the presence or absence of certain typical substances throws some light on the metabolism of the embryonic tissue.

**The water content of embryonic muscular and nervous tissues.**—It is well known that embryonic tissues have an exceptionally high water content. For the muscular parts Jakubowitsch<sup>3</sup> obtained figures as high as 99.4 per cent, diminishing gradually to 81 per cent toward the end of foetal life, the water content in the muscles of full-grown mammals being somewhat lower (75–80 per cent). In the nervous tissue (brain), also, the water content is higher during

<sup>1</sup> This research was conducted with the aid of a grant from the Carnegie Institution of Washington.

<sup>2</sup> BARDEEN: Contributions to the science of medicine, dedicated to W. H. Welch, 1900, p. 367.

<sup>3</sup> JAKUBOWITSCH: Archiv für Kinderheilkunde, 1893, xiv, p. 355.

fœtal life. Supplementing the analyses in the text-book literature, we can add the following estimations made on the brain substance of embryo pigs early in fœtal life. Distinctions between white and gray matter are not to be made at this period.

Size of embryo mm.	Water content of brain <sup>4</sup> (average, per cent).
50	90.3
75	90.2
100	90.7

In other organs there are similar differences between the fœtal and adult tissues. Thus an investigation of the liver of the pig showed:

Size of embryo mm.	Water content of liver <sup>5</sup> (average, per cent).
35	80.8
50	80.2
75	79.5
100	80.3
150	81.1
175	80.0
<i>Full-grown animal</i>	67.3

**Creatine in embryonic muscle.**—Krukenberg <sup>6</sup> detected creatine in the muscles of the fœtal calf at varying ages. Creatinine was not found,—an observation in accord with our own experience, and that of Grindley and Woods <sup>7</sup> on *fresh* meats.

After having demonstrated the presence of creatine in the embryonic muscles of the pig, we made an estimation in duplicate in the tissues removed from the back and pelvis of 265 mm. embryos. The directions of Van Hoogenhuyze and Verploegh <sup>8</sup> were followed in extracting the tissue and converting the creatine to creatinine for estimation by Folin's method with the Duboscq colorimeter.

<sup>4</sup> The estimations were made by drying the tissue to constant weight in a vacuum over calcium chloride. This usually required about fifteen days.

<sup>5</sup> The pulped tissue was weighed to tenths of a gram and dried to constant weight on a steam bath.

<sup>6</sup> KRUKENBERG: Untersuchungen aus dem physiologischen Institut der Universität Heidelberg, 1880, iii, p. 217.

<sup>7</sup> GRINDLEY and WOODS: Journal of biological chemistry, 1906-1907, ii, p. 310.

<sup>8</sup> VAN HOOGENHUYZE and VERPLOEGH: Zeitschrift für physiologische Chemie, 1905, xlv, p. 432.



The average creatine content thus ascertained was 0.03 per cent.<sup>9</sup> In the muscles of the full-grown pig Van Hoogenhuyze and Verploegh found about 0.45 per cent. These differences, noted at a late foetal period, cannot be due to differences in water content. Possibly the relative preponderance of connective tissue will account for them, or they may be associated with functional conditions. It is interesting to note in this connection that Dorner<sup>10</sup> has found the "total creatinine" content in young rabbits to be lower (0.3 per cent) than that of older animals (0.4 per cent).

**The purines of embryo muscle.** — Kossel<sup>11</sup> showed long ago that the embryonic muscle of cattle is distinctly richer in guanine than is the corresponding adult tissue. His figures obtained by older methods, without consideration of adenine, were:

	Guanine per cent.	Hypoxanthine per cent.	Xanthine per cent.
Embryonic muscle . . .	0.044	0.038	0.012
Adult muscle . . .	0.005	0.053	0.012

This difference is ascribed to the comparative richness of the embryonic tissue in nuclear material. During our attempts to ascertain whether the synthesis of nucleic acid in the embryo is accomplished with the same purine bases as are obtainable later from the tissue, 100 mm. pig embryos were examined. The heads and viscera were removed, and the remaining tissue comminuted and heated with 5 per cent sulphuric acid until the solution no longer gave the biuret reaction. The isolation of the purines was effected by Levene's method.<sup>12</sup> Thirty embryos, treated as described, yielded:

Guanine hydrochloride . . .	0.137 gm. ( <sup>13</sup> )	= 0.093 gm. guanine
Adenine picrate . . .	0.185 gm. ( <sup>14</sup> )	= 0.065 gm. adenine
Hypoxanthine nitrate . . .	0.057 gm.	= 0.029 gm. hypoxanthine

These substances, of course, represent the *total* purine content of the tissue. Some idea of the character of the *free* purines was

<sup>9</sup> These estimations were made by Mr. H. S. TURRILL.

<sup>10</sup> DORNER: Zeitschrift für physiologische Chemie, 1907, lii, p. 263.

<sup>11</sup> KOSSEL: Zeitschrift für physiologische Chemie, 1884, viii, p. 407.

<sup>12</sup> LEVENE: This Journal, 1904-1905, xii, p. 276.

<sup>13</sup>  $C_5H_5N_5O \cdot HCl \cdot 2H_2O$ ;  $H_2O = 16$ . 3 per cent: calculated, 16.1 per cent.

<sup>14</sup> M. p. 282° C.

obtained by examining the water extracts of about 5 kilograms of eviscerated pig embryos of 175–200 mm. length by the Krüger-Schmid method. 0.04 gm. hypoxanthine nitrate = 0.025 gm. hypoxanthine was obtained. The same quantity of adult pig muscle yielded:

Guanine hydrochloride . . . .	0.027 gm.
Hypoxanthine nitrate <sup>16</sup> . . . .	0.059 gm.

Krukenberg<sup>16</sup> long ago demonstrated the presence of free hypoxanthine in the muscles of foetal calves.

The preponderance of adenine and guanine in the entire tissue corresponds with the experience gained with other organs.<sup>17</sup> Free hypoxanthine is found in the embryonic as in the adult muscle. It is interesting to note that, according to Krukenberg,<sup>18</sup> in the selachians which contain an abundance of urea in their muscular tissue, the latter substance is also found in the embryonic forms.

**Lactic acid.**—The occurrence of lactic acid in muscle tissue has recently been carefully investigated by Fletcher and Hopkins.<sup>19</sup> They have shown that in freshly excised resting amphibian muscle only small amounts of lactic acid are found, — possibly not more than can be accounted for by manipulation prior to extraction. Lactic acid is spontaneously developed, under anaerobic conditions, in excised muscles. In the presence of oxygen, however, this survival development is inhibited, or lactic acid may be made to disappear. The evidence points to a relationship between lactic acid and intermediary muscular metabolism. We have sought for evidences of lactic acid in embryonic muscular tissue, by the familiar method of extraction with water, ether, and the formation of the zinc salt. Fifty-nine hundred grams of body tissue (muscle and connective tissue) of 175–200 mm. pig embryos yielded 0.0075 gm. zinc lactate. In a comparable experiment with fresh adult pig muscle, 5100 gm. yielded 0.302 gm. zinc paralactate ( $H_2O = 12.9$  per cent).

<sup>15</sup> This was united with that obtained from the embryos and analyzed.  $C_5H_4N_4O \cdot HNO_3 \cdot H_2O$ ; N = 32.1 per cent; calculated, 32.2 per cent.

<sup>16</sup> KRUKENBERG: Untersuchungen aus dem physiologischen Institut der Universität Heidelberg, 1880, iii, p. 217.

<sup>17</sup> Cf. This journal, 1907, xx, p. 102 ff.

<sup>18</sup> KRUKENBERG: Vergleichende-physiologische Vorträge, 1886, v, p. 314.

<sup>19</sup> FLETCHER and HOPKINS: Journal of physiology, 1906–1907, xxxv, p. 247.

**The lipoids of the nervous tissue.** — The lipid content of the embryonic brain has already been studied in foetal calves by Raske.<sup>20</sup> Our examination of the brain substance of the embryo pig at early ages corroborates his findings and need not be reported at length. Raske failed to find cerebrins, which are evidently associated with the medullary sheaths of the nerve fibres and conspicuous in "white matter." Since the sheaths do not develop until late in foetal or early in post-embryonic life, the absence of cerebrins is typical of the lack of white matter.<sup>21</sup> As Raske remarks, the embryonic brain evidently furnishes nervous tissue devoid of white matter, and may therefore afford a good source of pure "gray substance" for investigation. Following Koch's method,<sup>22</sup> *we were unable to detect cerebrins in the brain during foetal life.*

Raske found cholesterol present in the brains of the foetal calves examined. We have analyzed the brains of pig embryos at early ages by Ritter's method<sup>23</sup> with the following results:

Size of embryo.	Number of brains used.	Weight.	Dry solids.	Cholesterol.	
				gm.	per cent
50	260	158	15.4	0.375	2.4
100	120	204	22.0	0.967	4.4

The comparatively low content of cholesterol is likewise characteristic of the gray matter of the brain.<sup>24</sup>

#### SUMMARY.

Analytical data are presented giving further evidence of the comparatively high water content of embryonic tissues.

Creatine is present in the embryonic muscle, the content being considerably lower than in full-grown animals.

Among the purine bases obtainable from embryonic muscular structures, adenine and guanine preponderate as in other organs. Hypoxanthine is found *free* in the tissue, as in adult life.

<sup>20</sup> RASKE: *Zeitschrift für physiologische Chemie*, 1886, x, p. 340.

<sup>21</sup> Cf. KOCH's analyses of nervous tissues: *This journal*, 1904, xi, p. 303.

<sup>22</sup> KOCH: *Loc. cit.*, p. 323.

<sup>23</sup> RITTER: *Zeitschrift für physiologische Chemie*, 1901, xxxiv, p. 456.

<sup>24</sup> Cf. KOCH: *Loc. cit.*

Lactic acid can be separated from embryonic muscular tissue, but in exceedingly small amount.

The distribution of lipoids in the embryonic brain resembles that in "gray" matter. Cholesterol is present at the earliest periods, while cerebrins are entirely absent.

# CHEMICAL STUDIES ON THE CELL AND ITS MEDIUM. — III. THE FUNCTION OF THE INORGANIC SALTS OF THE PROTOZOAN CELL AND ITS MEDIUM.<sup>1</sup>

By AMOS W. PETERS.

[From the Zoological Laboratory of the University of Illinois.]

## CONTENTS.

	Page
I. The problem of the inorganic salts . . . . .	105
II. Method and results of experiments with distilled water . . . . .	106
III. The function of the salts in the life of the cell . . . . .	119
IV. Summary . . . . .	123
V. Bibliography . . . . .	124

## I. THE PROBLEM OF THE INORGANIC SALTS.

THE inorganic salts are constant constituents of the protozoan cell and of all the media in which these animals naturally occur. In this respect the protozoan and its environment do not differ from the mammalian cell surrounded by its salt media. The same problem of the function of the inorganic salts presents itself in both groups. Biological experiment has shown that the salts are necessary for the growth and development of the cell. In a previous paper (Peters :04) I have shown the close relation between the presence of these salts in the medium and the process of cell division in certain protozoa. Whether the ordinary metabolic functions of a cell can be continued in a medium of pure distilled water, *i. e.*, in the absence of an external supply of these salts, is a question which apparently will receive a different answer according to the cell under investigation. Without entering into details it may be said at once that for the protozoan cell, especially *Paramæcium*, the accounts given are conflicting. In the work here described this question has been further tested with positive result, although the information

<sup>1</sup> Read in the section on Comparative Physiology of the Seventh International Zoölogical Congress, Boston, Mass., August, 1907.

contributed from these experiments regarding the function of the inorganic salts is of an indirect and general character. The abstraction of salts by distilled water is simply an introductory step in the general problem of their function.

A few references to literature regarding the use of distilled water with *Paramæcium* may here be given. Jennings, '97, p. 272, found that *Paramæcia* "live in the distilled water for weeks," and he observed their increased sensitiveness to experimental treatment when the animals had been subjected to distilled water. No evidence is given regarding the condition of the water during the time the organisms continued to live in it. Miss Towle, :05, p. 221, in regard to distilled water, says: "Two further distillations made in glass rendered it perfectly harmless, and *Paramæcia* often lived in it without food for weeks at a time." No data are given pertaining to the condition of the water after the animals had lived in it for some time. Barratt, :05 a, p. 68, and :05 b, p. 78, kept *Paramæcia* in distilled water, whose origin is not described, for from three to twenty-four hours, in some cases changing the water before using the animals for experimental purposes. Greeley, :04, p. 10, included distilled water among the non-electrolytes which liquefy and presumably destroy protoplasm "through the absorption of water." According to Miss Towle, :05, p. 222, the water Greeley "used was not redistilled in glass." These references show that the action of distilled water, especially upon unicellular organisms, requires further investigation.

## II. METHOD AND RESULTS OF EXPERIMENTS WITH DISTILLED WATER.

The use of distilled water as a medium for living organisms has been frequent in biological experimentation. It is, however, evident, from the descriptions given, especially those dealing with the protozoa, that the conception of the physicist and the chemist of what is meant or should be meant by the term "distilled water" has not always been rigidly adhered to in biological work. It is manifestly erroneous to conclude from experiment that organisms continue to live in distilled water if the conditions of the experiment are not such as to exclude contamination of the water originally obtained by distillation, by the very presence in it of the objects of experiment. Since the chances for such contamination by the natural

processes of living cells are very great, the experimenter who draws the above conclusion cannot escape the obligation to demonstrate objectively the condition of the water during and after the period under consideration in which it has been inhabited by living organisms. Water may correspond to the description of distilled water at the moment of beginning an experiment, but it may be no longer in this condition after an interval of a minute. The liability to error is here so great that inference cannot be substituted for demonstration. Still more is this true in view of the fact that we possess very precise methods for the investigation of distilled water. The experiments here to be described were planned with the purpose of subjecting living protozoan cells to the continuous action of distilled water and of demonstrating the condition of this water during the course of the experiment. The result briefly stated was that the unicellular organisms so tested could not continue to live in water whose purity corresponded to the description to be given.

For physiological purposes the term "distilled water" may be taken to signify the purest water obtainable in contact with air of average purity. Absolute  $H_2O$ , necessarily prepared and used *in vacuo*, is not contemplated in these experiments. On the other hand, the extent to which absorption of both the gaseous and suspended normal constituents of an ordinary atmosphere is included ought to be indicated by some form of measurement rather than to be made a matter of definition. As a matter of fact, in all the physiological experiments with pure water thus far made, the essential principle involved was that the water described as distilled or pure should be free from all constituents not originating from a normal atmosphere. The principal contamination thus excluded consisted of dissolved solid matter, especially the inorganic salts. Indeed the motive for these experiments was almost always to determine the effect of the withdrawal of the salts which belong to physiologically normal conditions. This also has been the purpose in the experiments which I shall describe, and hence the definition of distilled water above given. The important point in my experiments is that the water is to be maintained in its condition of original purity, or its deviation therefrom is to be exhibited by measurement.

I shall next describe the preparation of the water here used. In view of the objective test to be applied at all stages of my experiments to the water in use, it is not necessary to review in detail here the literature on the preparation of distilled water by both chemists



and biologists. We need only refer to Kohlrausch und Holborn, '98, pp. 111-115, and the references there given, also to Bullo, '04, pp. 199-217; Lyon, '04, pp. 198-202; Locke, '95, pp. 319-331. Giving due weight to the results obtained by these investigators, I adopted Jena glass as a sufficiently safe material for my purpose, and under the conditions of my experiments for the distillation and storage of water. The apparatus for distillation consisted of a tin boiler connected with a condenser of Jena glass, and collecting flasks, also of the latter material. The connection between boiler and condenser was so made as to make the spurting over of liquid into the condenser practically impossible. The tin boiler was wrapped with asbestos except on its bottom. When the apparatus was first set up, in addition to ordinary chemical cleaning, it was subjected to a thorough steaming without other than air cooling of the condenser. At all times the quality of the water produced was regarded as the best evidence of the condition of the apparatus. A distillation was begun with the laboratory supply of ordinary once distilled water. The boiler was nearly filled, and then an excess of solid barium hydroxide was added. The orifice of the boiler for the introduction of water was left open until the whole content had been well boiled. It was then closed, and the distillate was collected from the condenser in Jena flasks, which were changed at intervals. From time to time the electrical conductivity of the distillate was determined by the Kohlrausch method, using unplatinized electrodes. The conductivity of the first portions distilled over was high, even higher than that of the original once distilled water. It was observed that it was necessary to boil away about one fourth of the original quantity in order to obtain water of low conductivity, *e. g.*, from 3 to  $1 \times 10^{-6}$  reciprocal ohms. The best water came from the latter half of the distillation, and the last fourth of the quantity placed in the boiler was not distilled. The taking of samples at frequent intervals for conductivity tests was never neglected, and the conductivity of the total quantity in a single Jena flask for storage was always taken, both at the beginning of the period of storage as well as immediately before use in experiments. The water was not stored more than a few days before use. The storage flasks were covered with inverted beakers and set away from dust and air currents. It was found that little or no deterioration occurred during storage. The water was permitted to drop a few inches from the end of the condenser into the collecting flask. The method of

collection and storage insured the aeration which was desirable for physiological experiment.

Water of determined quality having been prepared, the next problem was that of its application to living cells. In a former study, Peters, '04, pp. 449, 475-479, 503-504, I placed the organisms in the same test cell which was used to measure the electrical conductivity of the contained liquid, and I took these measurements at intervals of a few minutes. The result clearly showed that the distilled water with which the experiments began did not remain in that condition, but underwent a progressive contamination, as shown by the regularly increasing conductivity of the medium during a period of one hour. The control showed practically no change in its conductivity. The animals survived the experiment, which had been undertaken to test their permeability, and it is evident that this method is not well adapted to test the question whether the animals can live continuously in distilled water. In spite of the control the presence of the platinum may be harmful (Locke, '95) if prolonged. If the contaminated medium could be removed and replaced with sufficient frequency, and conductivity tests taken as frequently, the desired end could be accomplished. This method was easily applied by the use of a centrifuge. The apparatus employed was a hand centrifuge carrying four graduated glass tubes of 15 c.c. capacity each. Its rate of revolution was easily regulated to a known number of revolutions per minute, ranging from a few hundred to about two thousand. High speed was not desirable, owing to the considerable compression to which it subjected the animals in the bottom of the glass tubes. A rate of between 500 and 1000 revolutions per minute was found sufficient to so concentrate the objects used in the bottom of the glass tubes as to permit of pouring off 14 c.c. of the 15 c.c. contained in it without loss of animals, provided the pouring off was done immediately after stopping the centrifuge. If after centrifuging at a rate just sufficient to collect all suspended objects, whether animate or inanimate, in a gently compressed mass at the bottom, the tube was then allowed to stand at rest for some time, the living protozoa could be seen rising from the bottom like a cloud. The objects used, *Paramæcia* and *Stentor*, are visible to the naked eye. The débris of course remained at the bottom, and that portion of the medium containing the living animals could then be easily poured off into another clean glass tube of the same construction. This proved to be a most excellent method

of obtaining animals free from the *débris* naturally occurring with them in their culture liquid. For this purpose the first few centrifugings of an experiment were carried on with the animals in their original culture liquid, for they rise less easily after they have been treated with distilled water. But after any centrifuging in which moderate speed has not been exceeded the animals if still alive may be induced to rise from the bottom by gently rolling the tube between the hands. Thus, after a few centrifugings and by pouring from one tube into another, I obtained the animals free from suspended *débris* and in their original culture liquid. After the animals had become uniformly distributed by pouring or by rolling the tubes, a portion was examined very carefully for evidence of injury by the process itself of centrifuging. No evidence of injury could be obtained by optical examination. In fact, I have every reason to think, and still further evidence to adduce, that all the centrifugings to which the animals in these experiments were subjected produced no changes outside the limits of normal physiological variation of functional activities. Numerous observations of workers with these organisms indicate that they are of a most susceptible organization. I merely wish to assert that the kind of centrifuging I applied did not produce pathological specimens, although it may have influenced to a slight degree their normal physiological processes. In the record given below of these experiments there will always be given also the record of a control tube containing animals in their natural culture medium, freed from *débris*. This tube always received the same centrifugings in number, rate, and duration as the test specimens in distilled water. In fact, the control tube and those for the tests with distilled water were always carried together at the same time upon the centrifuge, which had four receptacles for them. These control tubes were always rolled or poured when the test specimens were subjected to the same treatment. It is scarcely necessary to add that neither corks nor fingers were allowed to come in contact with any of the media under experiment. These liquids came in contact only with glass, air, and the experimental animals. The animals of these control tubes were examined during and after the experiments, and were found wholly unchanged in so far as direct observation could determine. In fact, they lived in these same tubes for days after the experiments. Having prepared as above described a sufficient quantity of animals in their natural culture medium and free from *débris*, they were concentrated by the

same process of centrifuging so as to get a large number of organisms into a small volume. Of this liquid, in which they were uniformly distributed, portions were poured into the glass tubes of the centrifuge, one of the tubes being marked as the control, the others being destined for treatment with distilled water. At the beginning of an experiment the electrical conductivity was measured of the particular sample of distilled water which was to be used, and also of the original culture liquid from which the animals were derived. Numerous centrifugings then followed, as indicated in the tables below. After each centrifuging 14 c.c. of the 15 c.c. contained in a tube was immediately poured off from the test specimens, and this was replaced by 14 c.c. of the original distilled water of known conductivity. The portion poured off was immediately used for a conductivity measurement, provided the condition of the animals indicated the advisability of making such measurement. As soon as the tube had been refilled the animals were uniformly distributed as previously described, and then a small portion or the whole was poured off into a shallow flat-bottomed watch glass. An optical examination was now made with a Zeiss binocular and also with the compound microscope. Morphological detail could not be sought in the short time available between centrifugings. Only the gross effects were observed, and the attention was directed especially to changes of a physiological character such as those of movement. The changes observed are recorded in the following tables. The control tubes, which ran parallel with the tests, were treated as above described.

In the following tables the first column shows the number of the centrifuging in serial order. The second column records the time at which the respective centrifuging ceased. In the third column is given the electrical conductivity in reciprocal ohms  $\times 10^6$ . The conductivity given opposite centrifuging No. 0 in each table is that for the distilled water which was used throughout the given experiment to refill the tubes after pouring out 14 c.c. of their content. In the fourth column is given a rough description of the condition of the animals which were examined at the intervals indicated. The conductivity measurements were made at 18° C.

TABLE I.  
PARAMÆCIA AND DISTILLED WATER.

No. of centrifuging.	Time.	Electrical conductivity $\chi \times 10^6$ .	Condition.
0	....	2.3	
1	10.15	..	
2	....	..	
3	....	..	
4	10.30	..	No visible change.
5	10.33	..	
6	10.35	..	Locomotion slow. Swelled in middle.
7	10.45	..	
8	10.48	..	
9	10.50	8.9	Enlarged vacuoles. Disintegration. Many dead. Those living show slow locomotion.
10	10.55	..	
11	11.00	..	Very few alive. Much swelled.
12	11.05	..	All dead. Most forms disintegrated.
13	11.10	..	All disintegrated.
14	11.15	7.9	

The control examined at centrifugings Nos. 6, 9, and 14 showed all the animals alive and normal. The animals were raised in culture No. 6-3-8, the chemical data of which I have described in some detail in Peters, :07, pp. 443-477. The observed conductivity of this culture liquid was  $\chi = 643 \times 10^{-6}$ . Methyl orange alkalinity = 3.95 c.c. of 0.0102 m. HCl for 5 c.c. of culture liquid.

TABLE II.  
PARAMÆCIA AND DISTILLED WATER.

No. of centrifuging.	Time.	Electrical conductivity $\times 10^6$ .	Condition.
0	....	3.0	
1	10.40	..	
2	....	..	
3	....	..	
4	10.50	..	
5	10.55	..	
6	11.00	..	No visible change.
7	11.15	..	
8	11.20	..	
9	11.25	4.5	Locomotion slowed.
10	11.30	..	
11	11.32	..	
12	11.35	..	Wabbling movement. Little locomotion. Enlarged vacuoles. Swelled in middle.
13	11.40	..	
14	11.45	..	
15	11.46	..	
16	11.48	4.5	No locomotion. Little wabbling. Much swelled. Enlarged vacuoles.
17	1.20	9.0	All dead. Probably died sooner. Forms half disintegrated.

The conductivity of No. 17 was taken with the dead animals in it. Control was alive and normal. Conductivity of original culture liquid =  $616 \times 10^{-6}$ . Methyl orange alkalinity = 3.9 c.c. of 0.0102 m. HCl for 5 c.c. of culture liquid.

TABLE III.  
PARAMÆCIA AND DISTILLED WATER.

No. of centri-fuging.	Time.	Electrical conductivity $\times 10^4$ .	Condition.
0	....	2.8	
1	9.35	..	
2	9.39	..	
3	9.43	..	
4	9.45	..	No visible change.
5	9.47	..	
6	9.52	..	Locomotion slightly sluggish.
7	9.55	..	
8	9.58	..	Locomotion slightly sluggish.
9	10.09	3.5	
10	10.12	..	Locomotion sluggish. Vacuoles enlarged.
11	10.15	..	
12	10.18	8.0	All forms still whole. Few motionless. Vacuoles enlarged.
13	10.25	..	
14	10.28	..	Same as No. 12.
15	10.33	..	
16	10.37	8.0	Many dead. Those living show wabbling movement (not locomotion).
17	10.47	..	
18	10.50	..	About same as No. 16.
19	10.55	..	
20	11.00	..	
21	11.15	..	Some disintegration. Many dead.
22	11.20	..	
23	11.25	..	Same as No. 21.
24	11.30	..	
25	11.35	..	Many disintegrating. Living ones inactive.
26	11.37	..	
27	11.40	..	
28	11.42	..	Very few alive.
29	11.45	..	
30	11.48	7.3	All dead.

Control was alive and normal.



TABLE IV.  
STENTOR CERULEUS AND DISTILLED WATER.

No. of centrifuging.	Time.	Electrical conductivity $\chi \times 10^6$ .	Condition.
0	....	2.7	
1	10.00	..	
2	10.02	..	Settled rapidly to bottom.
3	10.05	..	No further change.
4	10.10	..	
5	10.15	..	Sluggish locomotion. No form changes.
6	10.18	..	
7	10.22	..	Same as No. 5.
8	10.27	..	
9	10.30	..	Few dead. Some disintegration. Sluggish.
10	10.36	..	
11	10.40	..	About one fourth of the number dead. Disintegration. Sluggish.
12	10.44	..	
13	10.47	..	Many dead. Living are of irregular outline.
14	10.54	..	
15	10.57	..	Few living. Nearly all disintegrated.
16	11.05	..	Very few living. Disintegration.
17	11.10	..	About 12 still living.
18	11.15	..	About 6 still living.
19	11.20	..	About 4 still living.
20	11.30	..	About 4 still living.
21	2.00	4.0	All dead. Probably died sooner.

Control was alive and normal. Conductivity of the original culture liquid was  $\chi = 565 \times 10^{-6}$ . Methyl orange alkalinity = 3.9 c.c. of 0.0102 m. HCl for 5 c.c. of culture liquid.

TABLE V.  
STENTOR COERULEUS AND DISTILLED WATER.

No. of centrifuging.	Time.	Electrical conductivity. $k \times 10^6$ .	Condition.
0	....	3.0	
1	10.30	..	
2	10.35	..	No visible change.
3	10.40	..	
4	10.44	..	Enlarged vacuoles. Sluggish.
5	10.47	..	
6	10.50	..	Few dead. Sluggish.
7	10.58	..	
8	10.59	..	Half the number dead. Many disintegrating.
9	11.02	..	Sluggish.
10	11.05	..	Many dead. Disintegration. Sluggish.
11	11.07	..	
12	11.10	..	Three fourths of the number disintegrated.
13	11.12	..	
14	11.15	..	Nearly all disintegrated. Few alive.
15	11.20	..	All but very few disintegrated.
16	11.25	..	All but very few dead.
17	11.30	..	All dead. Nearly all disintegrated.
18	11.35	8.0	All dead. All disintegrated.

Control was alive and normal.

It should be noted that the glass tubes in which the animals were centrifuged and the watch glasses in which they were kept for periods of time to be reckoned in very few minutes were not made of Jena glass. However, the results of the experiments are not vitiated by this fact. Control experiments were made with the same glass vessels and with the same distilled waters. These control tests extended over longer periods than the experiments with animals.

No contamination of the water could be detected by the conductivity test. All the glass vessels used were old, had been frequently cleaned with sulphuric bichromate and other agents. It is well known that the resistance of the surface of good glass becomes improved with continued use. No appreciable contamination of the water occurred under the conditions of these experiments, and especially is there no extraneous source for the emanation of toxic constituents. Whatever effects have been produced must be attributable to the pure condition of the water itself, *i. e.*, to the absence of the ordinary constituents of water or of the original culture liquid.

As shown by the tables just given, the effect of treating the free-living cells of *Paramæcium* and *Stentor* with distilled water in the manner described is the destruction of the cell within a short period of time. With *Stentor* I obtained practically the same result in a former investigation (Peters, '04, pp. 476, 504), but the methods then used involved the objectionable condition of leaving the animals in contact with their own excreted salts. Hence the result depended largely upon the ratio of the number of animals to the volume of water, as is well shown in the experiments just referred to. For *Paramæcium* my result is contrary to the several statements to be found in the literature on this subject. However, from the descriptions given it is evident that in general the authors of these statements mean that after having placed *Paramæcia* in a quantity of distilled water the animals continued to live in that same quantity of water or in a few changes made at longer intervals than those in my experiments. Furthermore, data from chemical or physical tests regarding the quality of the water to be so used are most frequently absent. In any case the proof for the proposition that a given cell can or cannot continue to live in pure aerated water is defective unless the method used meets the condition of possible contamination of the medium by the metabolic activities of the cells living in it for even a short time. In the experiments recorded in Tables I-V each process of centrifuging diluted the liquid remaining over from the preceding centrifuging to 15 volumes. Of the 15 c.c. contained in a tube 14 c.c. were removed and replaced by 14 c.c. of the distilled water of known quality. The number of centrifugings shown by the tables ranges from 14 to 30, and from this it is evident that the dilution of the original salt content soon becomes very great if the initial quantity was not too large. All the animals used in these experiments came from similar cultures

which were raised and examined according to the descriptions given in my previous papers (Peters, :07 a and :07 b), and which showed the usual course of chemical and biological events. Nearly the whole salt content was measurable as methyl orange alkalinity, and this did not differ much in the cultures whose records are given in the tables. For the discussions of this paper I shall adopt, as a type of all these cultures, the one numbered 6-3-8 which furnished the *Paramæcia* for Table I. I shall make use of the chemical and physical data of this culture which may be found in tabular form in a former paper (Peters, :07 a, pp. 467 and 476). From these data it appears that practically the whole salt content consisted of alkali bicarbonate + alkali-earth bicarbonate =  $5.46 \times 10^{-3}$  gram molecules per litre, *i. e.*, the culture liquid is about a 0.0055 molecular solution. By the method of the experiments recorded in Tables I-V of this paper, this concentration of salts would be diluted to 15 volumes after the first centrifuging, to  $15^2$  volumes after the second, etc. It is evident that at this rate a smaller number of centrifugings than are given in any of the Tables would reduce the concentration of the salts to a point at which the amount of the conductivity due to them would be negligible in comparison with the conductivity of the distilled water used for dilution. This method of removing the natural salts of the culture medium may be regarded as practically perfect. If now the values for the conductivity measurements be examined, it appears that in none of the experiments was it possible, even by the rapid changes of medium shown by the tables, to keep down the conductivity to a point as low as that of the distilled water applied at each interval. The increase in conductivity after a few centrifugings was due to substances originating from the cell bodies, not from the original culture liquid nor from the apparatus. After some of the animals had died and disintegrated, this factor became marked. But preceding the death of the animals there was a period when the harmful effects of the change of medium were evident from the behavior of the animals. These effects began to show at a few centrifugings earlier in the case of *Stentor* than in *Paramæcium*. The former is more sensitive to most environmental conditions than the latter, according to various other tests which I have made. It is clear that the fatal effect in these experiments is due to contact with pure water as a medium when applied under certain conditions.

In addition to the continued purity of the water the element of

time must be considered. If the change from culture liquid to pure water be made as rapidly as in these experiments, a fatal effect ensues. On account of the conception I have formed of the mode of action of the pure water, I would expect the same effect to occur even if the changes occupied a longer period of time. Their death from a continuously pure medium would probably occur sooner than death from starvation. I have kept *Paramæcium* and *Stentor* under the same cover-glass in a moist chamber in the *same original* quantity of pure distilled water for a period between one and two weeks before death ensued. The animals became exceedingly transparent, making fine objects for microscopical examination. No evident abnormality was observed. Of course this was not a test of the ability of the animals to live in continuously pure, aerated, distilled water. The time required to produce the effects of pure water obviously depends upon several conditions, among which we may mention (1) the number of the animals, (2) the volume of the water, (3) the frequency of its renewal after contamination, (4) the physiological condition of the organisms.

Study of the changing condition of the animals during the course of the experiments shows more or less uniformity in these changes. After the first few centrifugings the first noticeable effect was a retardation of locomotion. This difficulty increased until they became motionless. Early in the experiment there also appeared a noteworthy enlargement of vacuoles, sometimes becoming so great, especially in the middle region of *Paramæcia*, as to break the animal in halves, which then rapidly disintegrated. This latter process of disintegration was a third phenomenon of uniform occurrence and brought the experiment to a close. While swelling of the whole cell bodies of either *Paramæcium* or *Stentor* was not marked, the enlargement of vacuoles to such extent as to cause their breaking through the bounding cell wall at some point on its surface, and so to begin the process of disintegration, was always so pronounced as to attract the attention of the observer.

### III. THE FUNCTION OF THE SALTS IN THE LIFE OF THE CELL.

In a previous paper (Peters, '04, pp. 480-488), I have given an explanation of the mode of action of pure distilled water upon the cell of a free-living protozoan. In the present discussion I shall adopt essentially the same explanation. However, in consequence

of the almost continuous removal of the contaminated water, the experiments recorded in this paper gave better evidence than was formerly obtained regarding the water content and the vacuolation of the protoplasm. This effect is also more easily observed in *Paramecium* than in *Stentor*, to the latter of which the earlier experiments were confined.

To describe how the distilled water produced its effects, and what the function is of the salts normally present in the natural culture medium or in the protoplasm of the cell, it is necessary to know what physical and chemical conditions prevail in this complex system. In the present state of our knowledge we have an insufficiency of detail that prevents us from touching the essentials of the problem intimately, and explanation is necessarily superficial. With such data as I have obtained I should attempt to explain the phenomena of the preceding experiments as due, not to the influence of any one predominant factor, but as the result of the disturbance of the natural equilibrium which normally exists between the various physical and chemical conditions of the protoplasm. The most important factor in our conception of conditions in the cell is that of its continuous dynamic activity in relation to the maintenance of its identity. In other words, each kind of protoplasm is a complex system of chemical substances whose continuous physical and chemical interaction is so equilibrated as to preserve its morphological and physiological individuality. It is from this standpoint that its problems must be approached, even though we are as yet unable to analyze the complex far into its details.

For the physical condition of the protoplasm of the protozoan cell we shall adopt the conception of Bütschli, '92, pp. 139-158, that we have in the cell content a more and a less viscous liquid, the latter of which is enclosed by a framework of the more viscous substance. The cell wall may be regarded as the outer boundary of this framework. The more watery liquid is thus entirely enclosed in the little meshes of the framework or in the larger vacuoles, which do not differ in nature from the smaller meshes. Metabolic exchange between the cell and its environment must take place through the framework, which functions as a colloidal osmotic membrane. The content of both vacuoles and of the framework must be regarded, in the light of our present knowledge, as a mixture of both crystalloidal and colloidal substances, subject to continual changes from either condition into the other. A proper conception of these

conditions must reckon with the ideas of Loeb, :00, p. 327, on ion-proteids; also the developments of Pauli, :03, pp. 225-246, and of Spiro, :04, pp. 300-322, regarding the relation of salt ions to the colloidal proteins; also of Pauli, :07, pp. 143, 146, and of Friedenthal, :04, pp. 60-61, regarding the H and OH charge of native proteins.

We may now consider the process of exchange between the cell and its medium. As the natural salt containing culture medium was replaced in the experiments by distilled water, salts or their radicals originating from the cell appeared in it. Hence the cell wall and the viscous framework are permeable in the outward direction at least to the cellular salts or their ions. It may be noted here that the cell does not acquire these salts necessarily by inward permeation. They may be so obtained, but they may also be supplied by the ingested food matter. It is probable that the permeation of the salt ions differs in rate. In any case the permeation of the salt ions is slower than that of the distilled water, for observation showed a swelling of the vacuoles among the pathological phenomena. Had the wall or the framework been impermeable to the salt ions, there would have been much more evidence of an accumulation of water within the cell and much plainer evidence of the part played by mechanical rupture in the destruction of the cell. The moderate effect of this kind which was obtained is in harmony with the conclusions reached by most observers of physiological osmosis that the mechanical effect produced is of minor importance. The disturbance of salt balance and the relation of salt ions to the protoplasmic proteins are facts which point to a deranged metabolism as the essential factor. It is highly probable that continued contact with distilled water progressively altered the condition, and so increased the permeability, of the colloidal wall and framework. The smaller the difference between the rate of permeation of the water and of the salts, the less would any mechanical effect of osmosis come under observation as a resulting change of volume. In this connection it should be noted that when any cells began disintegration by rupture of an enlarged vacuole, that event was preceded by marked pathological effects shown in the behavior of the cell. These effects may have a dependence upon a structural as well as an accompanying metabolic change due to the fact that an increased water content produces alterations in the colloidal state of the protoplasmic proteins. In a former work (Peters, :04) I showed that



the injurious effect of distilled water can be prevented by dissolving in it appropriate salts or milk sugar. The latter, as judged by its effect upon the rate of division (Id. pp. 473-475) and upon the general behavior of *Stentor coeruleus*, makes a solution practically indifferent with reference to the metabolism and structure of the cell. The experiments with milk sugar add weight to the theory above described that alteration of the structure of the cell wall leading to increased and abnormal permeability is the initial factor in the destructive action of distilled water.

The salts are held by the cell with varying degrees of intensity. A portion of the salt content is easily withdrawn and with little immediate consequence to its normal functioning. This was illustrated in the above experiments, when the animals were transferred from a culture medium, known to have a salt content equal to at least a 0.0055 molecular solution (Peters, :07, p. 467), to pure distilled water. The water thereupon became contaminated sufficiently to permit the cells to live, provided the contaminated medium were not removed. As above stated, it is this kind of experiment which is usually meant when the statement is made that these animals continue to live in distilled water. There are several methods by which such an experiment can be conducted so that the contamination originating from the culture liquid is negligibly small compared with that coming from within the cells themselves. This loosely held portion of the salts may be regarded as subject to no stronger an affinity than that between the solvent and its solute. Its maximum quantity represents the limits of physiological variation. No doubt its withdrawal disturbs the internal equilibrium of chemical and physical changes and necessitates some readjustments. But if the experiments be continued as represented in the above tables of results, a further quantity, beyond the physiological limit, comes out, as is evident, respectively, from the conductivity tests and the observed pathological condition of the animals. Whether or not this second portion originates directly from ion-protein compounds of the protoplasm, we do not know. In the light of our present knowledge it is highly probable that these compounds are the essential seat of disturbance when cells of any sort are brought to destruction by means of distilled water. The salts or their radicals thus firmly combined with the protein molecules are examples of the greatest degree of affinity with which any of the salts are held by the cell. Between this intensity of affinity and that of the most

easily abstracted portion above described there probably exist all gradations of intensity. The study of the dynamics of reactions in crystalloidal and colloidal solutions has shown that the quantity of each of the different combinations represented by these different intensities would be in equilibrium with all the salts and other salt compounds present. A disturbance of this chain of equilibria anywhere along its course would result in a disturbance of the equilibrium of the entire system. This could occur internally by any abnormal change in metabolic reactions, or externally, as in these experiments, by the removal of the more easily withdrawn portion of the salts. It is probable that the initial effect is physical, consisting in an alteration of the colloidal condition of the proteins, which is readily produced by a change in the proportion of the salts. When this has proceeded beyond a limit, the protoplasmic chemical system becomes unbalanced, resulting in its self-destruction.

#### IV. SUMMARY.

These experiments were made to test the effect of the entire absence of salts from the external medium of the protozoan cell brought about by placing the animals in pure distilled water. They differ from other experiments for the same purpose in that the water used was maintained in a condition of almost continuous purity. This was accomplished by frequently changing the contaminated medium for a fresh portion of the original pure medium. Pure distilled water was prepared by redistillation of ordinary distilled water from Jena glass. All contact with metal anywhere in the experiments was avoided. The quality of the water was determined by measuring its electrical conductivity, and only water of low conductivity was used. Protozoa were subjected to the practically continuous action of water of this degree of purity, and in a condition of aeration, by frequently renewing the medium. By the use of a centrifuge it was possible to separate the greater portion of the medium, about 14 c.c., from the remaining 1 c.c. which contained the animals. To the residue was added 14 c.c. of the original pure water. The animals were then uniformly distributed, and the whole process was repeated at short intervals a sufficient number of times. Control tubes carried upon the same centrifuge and subjected to the same whirling and redistribution, but without change of medium, showed that the animals lived through the control experiment with-

out injury. Conductivity tests made upon the removed portions of water showed that it did not remain in the condition of distilled water. The animals repeatedly contaminated the water, even after numerous renewals of their medium had been made. They were unable to maintain life in a medium which was continuously kept as near as possible to the condition of pure aerated distilled water. Locomotion was retarded and finally ceased, the forms of the animals then disintegrating. Vacuoles were enlarged, and in some cases the entire form swelled. Distilled water progressively alters the character and permeability of the cell wall, which then permits the outward diffusion of the salts of the cell. Milk sugar and low concentrations of various salts dissolved in distilled water prevent its injurious action. Withdrawal of a portion of the salts is harmless. Excessive abstraction of salts is destructive, by disturbing the equilibrium of distribution of salts between the colloidal protoplasm and the liquids of its meshes. Primarily the physical condition of the colloidal proteins is altered by the qualitative or quantitative change in salts. In consequence chemical processes no longer maintain the equilibria of living protoplasm, and destruction of the system results.

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# ACAPNIA AND SHOCK.<sup>1</sup>—I. CARBON-DIOXID AS A FACTOR IN THE REGULATION OF THE HEART-RATE.

BY YANDELL HENDERSON.

(WITH THE COLLABORATION OF MARVIN McRAE SCARBROUGH, FELIX PERCY CHILLINGWORTH, AND JAMES RYLE COFFEY.)

[From the Physiological Laboratory of the Yale Medical School.]

## CONTENTS.

	Page
I. Origin of the problems . . . . .	126
II. Acapnia as the cause of surgical shock . . . . .	128
III. Carbon-dioxid as a hormone . . . . .	130
IV. Cardiac tetanus . . . . .	142
V. Acapnial shock under artificial respiration . . . . .	143
VI. Artificial regulation of the heart-rate . . . . .	147
VII. Shock and its prevention under the Sauerbruch-Brauer method of respiration . . . . .	150
Summary . . . . .	155

## ORIGIN OF THE PROBLEMS.

IN major surgical operations a pulse of extreme rapidity and correspondingly small amplitude is a dangerous symptom. Indeed a progressive increase in heart-rate up to extreme tachycardia is one of the features in the development of at least one form of shock. Yet the surgeon is often practically helpless when confronting such a progressively rising pulse, since he cannot intelligently counteract a process if he is ignorant of its cause. In the course of a series of experiments involving extensive operative procedures upon dogs we experienced a large number of failures.

<sup>1</sup> Preliminary reports upon various parts of this subject were presented by the writer before the Physiological Section of the British Medical Association in August, 1906 (published in the British medical journal, 1906, ii, p. 1812), and before the American Physiological Society in December, 1905, December, 1906, and May, 1907 (This journal, 1906, xv, p. xviii; 1907, xviii, p. xv; and 1907, xix, p. xiv). Portions of the subject matter of this paper were presented in the theses for the M.D. degree by F. P. CHILLINGWORTH and J. R. COFFEY, to the Faculty of the Yale Medical School, June, 1907.

Tachycardia and then shock developed. As this result seemed to us inexplicable by any principles of physiological reaction at present recognized, we were stimulated to discover, if possible, its hidden cause.

It is well known that healthy dogs are with difficulty brought into a condition of shock. Violent procedures, such as cauterization of a large area of the peritoneum,<sup>2</sup> crushing of a limb, and other forms of intense and prolonged stimulation of many afferent nerves, have generally been found necessary to induce this condition. Even under such treatment a dog which has received a dose of morphine and is maintained under moderate anaesthesia will not infrequently survive for many hours. The primary purpose of the series of experiments, among which occurred the failures to be here analyzed, was the study of the normal activity of the dog's heart under conditions as nearly physiological as possible. The utmost care was exercised therefore to avoid such injuries and stimulations. Yet during our earlier experiments we repeatedly saw the animals die in shock after passing through all the typical stages of this condition within two hours after the beginning of the experiment, and occasionally even within a much briefer period. These cases were the more enigmatic because other animals, which had received a precisely similar treatment, — so far as we were then aware, — maintained for many hours the normal slow heart-rate and high blood pressure requisite for the observations which were the primary object of the experiments.

Before the completion of this series of experiments these difficulties had been so far overcome that we could at will induce or prevent the occurrence of shock. Moreover the method employed to this end proved to be highly effective also as a means of varying the heart-rate. By this method we were enabled to regulate the heart to any desired rate from 40 or fewer up to 200 or more beats per minute. The method was very simple. It depended on the manipulation of the hand bellows with which artificial respiration was administered and on the adjustment of the escape vent in the side of the cannula tied into the trachea. As the pulmonary ventilation was increased or diminished the heart-rate was correspondingly accelerated or retarded. This method was utilized successfully on a large number of dogs, in which the behavior of the heart at various rates of beat was recorded. Since the results

<sup>2</sup> Cf. T. SOLLMANN: *This journal*, 1907, xx, p. 74.

thus obtained were published,<sup>3</sup> our attention has been devoted to investigations on the causal relations between the method and its results. The scope of these investigations has widened until they cover a great part of the entire subject of surgical shock and touch on a number of related topics. They will require a series of papers for detailed presentation. The hypothesis to the demonstration of which the entire series will be directed is as follows:

#### ACAPNIA AS THE CAUSE OF SURGICAL SHOCK.

From the data to be presented in this series of papers it appears that the  $\text{CO}_2$  content of the blood exercises regulative influences upon the heart-rate, upon vascular tonus, upon the peristalsis of the alimentary canal, upon the mental condition and upon a number of other functions of the body to a degree, so far as we can find from the literature, hitherto in great part not demonstrated. These data show that even a slight reduction in the  $\text{CO}_2$  content of the arterial blood causes a marked quickening of the heart-rate. Further reduction induces an extreme tachycardia, complete cessation of peristalsis, failure of many reflexes, and coma. If an extreme reduction of the  $\text{CO}_2$  content of the blood is effected very rapidly the heart comes into a state bordering on tetanus. This cardiac tetanus practically abolishes the pumping action of the heart. Arterial pressure falls therefore; and death results.

If the reduction in the arterial  $\text{CO}_2$  is less extreme but is maintained for a considerable time (an hour or more according to the extent of the reduction), so that the tension of  $\text{CO}_2$  in the venous blood and in the tissues is greatly reduced, symptoms and conditions result which are similar in many respects to those occurring in mountain-sickness, and are apparently identical with those of surgical shock. Arterial pressure falls to a very low level; and if the condition is continued the circulation fails. This fall is not due merely to tachycardia, for the heart-rate in the later stages is not always extremely rapid, but is caused by a loss of tonus in the peripheral veins and capillaries, and by the consequent stagnation of the blood in these vessels. The mental condition of the subject is comatose. The reflexes are greatly reduced in responsiveness. Vigorous stimulation of afferent nerves causes no rise of

<sup>3</sup> Y. HENDERSON: This journal, 1906, xvi, p. 325.



arterial pressure. This condition of the nervous system and the stage of excitement through which it develops, are not due primarily to the fall of arterial pressure. They precede the fall. Although the coma is of course intensified by a low pressure, it may occur to a considerable extent coincidently with the high pressure of the earlier stages.

The respiration, when the subject is left to breathe naturally, becomes very shallow. It is liable to pass into apnoea. This condition is the direct effect of the reduced  $\text{CO}_2$  tension in the respiratory centre.

The reduction of  $\text{CO}_2$  leading to these conditions can be effected by excessive artificial respiration. It can be effected by allowing the subject, after the thorax has been opened, to breathe by the Sauerbruch-Brauer method an excess of fresh compressed air. To a certain extent the conditions above described can be induced by a normal man upon himself by voluntarily forced respiration. They result to a considerable extent from the hyperpnoea incident to the stage of excitement of incomplete anaesthesia. The hyperpnoea induced by vigorous and prolonged stimulation of afferent nerves — a condition which, in an animal or man not anaesthetized, would be one of extreme pain — is an effective procedure for the reduction of the  $\text{CO}_2$  content in the blood. Morphine and complete anaesthesia counteract the development of shock by quieting the respiration. Diminution of the dead-space of the respiratory tract by tracheotomy removes an important natural check on excessive pulmonary ventilation. The expression "excessive" in this and similar connections is used to indicate the maintenance of a sub-normal tension of  $\text{CO}_2$  in the alveolar air.

Exposure of the abdominal viscera to the air so as to allow a free exhalation of  $\text{CO}_2$  from the surface of these organs induces, even when no further operation is performed, and when both cooling and drying are prevented, an extreme congestion in the part exposed. This congestion is relieved by placing the part in saline saturated with  $\text{CO}_2$ . If a general abdominal congestion has been thus induced and is then followed by even a moderate hyperpnoea through the stimulation of an afferent nerve, the subject passes within a few minutes into shock.

In the production of these conditions variations in the oxygen content of the blood appear to play little, if any, part. Artificial regulation of the heart-rate by the method which we have employed

appears to depend upon the regulation of the  $\text{CO}_2$  tension of the air in the pulmonary alveoli, and thus upon the extent to which  $\text{CO}_2$  is ventilated out of the blood in its passage through the lungs. The condition of shock which results in man from extreme physical suffering is to a large extent, we believe, caused by the reduction of the  $\text{CO}_2$  content of the blood and tissues below the normal tension by the violent and prolonged hyperpnoea induced by the stimulation of afferent nerves. The peculiar liability of laparotomy to result in shock is, we believe, due to the exhalation of  $\text{CO}_2$  from the parts exposed. Out of a very large number of experiments involving extensive and prolonged operations the symptoms of shock as above described have never developed in a single case in which any considerable loss of  $\text{CO}_2$  below the normal was prevented.

In a number of experiments in which shock had been induced, restoration of  $\text{CO}_2$  to the tissues and blood (or rather the maintenance of a condition which permits the tissues rapidly to restore their  $\text{CO}_2$ ) proved effective in inducing a rapid recovery. Consciousness and the normal reflexes returned simultaneously. The intestines recovered their automatic motility. Such a restoration is most easily accomplished by infusing saline saturated with  $\text{CO}_2$ , and simultaneously increasing the dead-space of the respiratory tract by causing the subject to breathe through a tube of about double the diameter of the trachea and 50 to 100 centimetres' length. Under all conditions, except during hyperpnoea, in which condition the cardiac activity is increased sympathetically with the respiratory excitement, and to a certain extent even in this condition, the heart-rate can be kept down and the development of shock prevented by the use of such a tube. In the absence of respiratory excitement the heart-rate is an index which varies inversely as the  $\text{CO}_2$  content of the arterial blood.

#### CARBON-DIOXID AS A HORMONE.

In discussing the subject of the "Chemical Co-ordination of the Functions of the Body" in the "*Ergebnisse der Physiologie*," Bayliss and Starling have given the first place to carbon-dioxid as the "hormone" or chemical regulator of respiration.<sup>4</sup> From

<sup>4</sup> *Ergebnisse der Physiologie*, 1906, Fünfter Jahrgang, Erste Abteilung, p. 664. For a general discussion in English of the subject of Chemical Correlation, see E. H. STARLING, *Lancet*, 1905, ii, pp. 339, 423, 501, and 579.

the extensive literature of respiration and from the more meagre and less definitely determined knowledge regarding the influence of  $\text{CO}_2$  in regulating other functions of the body we shall here cite only such points as appear to bear directly on the conclusions stated in the preceding section.

The observations of Haldane and Priestley<sup>5</sup> have afforded crucial evidence that "the regulation of the rate of alveolar ventilation in breathing depends, under normal conditions, exclusively on the  $\text{CO}_2$  pressure in the respiratory centre." In their experiments normal men were subjected to a wide variety of conditions in respect to the oxygen tension in the air breathed, — variations up to a full atmosphere of oxygen and down to only 13 per cent of an atmosphere partial pressure of this gas, — without the respiratory movements showing any reaction to these variations, and without the subjects themselves perceiving any alteration in their own condition. On the other hand, when the subjects were exposed to a wide variety of conditions in respect to barometric pressure and to the  $\text{CO}_2$  content of the air breathed, the respiration was found to vary in such perfect adjustment to these conditions that the tension of  $\text{CO}_2$  in the alveolar air was maintained nearly constant. Not only is the respiratory centre exquisitely sensitive to any rise in  $\text{CO}_2$  pressure, — a rise of 0.2 per cent of an atmosphere in the alveolar air being, for instance, sufficient to double the amount of the alveolar ventilation during rest, — but *the respiratory centre is almost equally sensitive to even a slight reduction in the tension of  $\text{CO}_2$  below the normal* (about 5.5 per cent of an atmosphere). Thus a brief period of voluntarily forced respiration in man is followed automatically by an interval of apnoea. As evidence that this apnoeic pause is due to the temporary lowering of the  $\text{CO}_2$  tension of the arterial blood below the threshold exciting value for the respiratory centre, Haldane and Priestley find that in man it does not occur when the air forcibly respired contains  $\text{CO}_2$  of nearly the same tension as that of the alveolar air.

It has long been known that vigorous artificial respiration is followed by a period of apnoea. In a review of the earlier investigations on this subject Miescher<sup>6</sup> pointed out that this pause might consist of two conditions, which he termed respectively

<sup>5</sup> HALDANE and PRIESTLEY: *Journal of physiology*, 1905, xxxii, p. 225. See also FITZGERALD and HALDANE: *Journal of physiology*, 1905, xxxii, p. 486.

<sup>6</sup> MIESCHER-RUSCH: *Archiv für Physiologie*, 1885, p. 355.

apnoea spuria and apnoea vera. The former is the result of the stimulation of the afferent nerve endings of the vagi in the lungs, by which the respiratory centre is temporarily inhibited. The latter is the effect upon this centre of the blood which is over-aerated by the excessive pulmonary ventilation. The changes in the blood might consist in an over-oxygenation or in a reduction of its content of  $\text{CO}_2$ . The first of these alternatives is rendered untenable by the fact that apnoea is not induced, indeed the rate and depth of respiration remain unaltered, when the subject breathes pure oxygen. In this case the alveolar tension of oxygen is increased far above the maximum attainable when the lungs are ventilated merely with air, — no matter how rapid the ventilation. Thus, as Miescher concluded, and as the recent investigations of Fredericq,<sup>7</sup> of Mosso,<sup>8</sup> of Weil,<sup>9</sup> and of Haldane and Priestley<sup>10</sup> have verified, apnoea vera is caused by the diminution in the  $\text{CO}_2$  of the arterial blood.

This conclusion as to the much disputed causation of apnoea is important in connection with the matters to be discussed in the present paper, because we have employed as the means of inducing tachycardia and shock the same procedures as those which induce apnoea vera. The facts and reasoning which have led previous writers to this explanation of apnoea vera may therefore be adduced with equal validity in support of the view that tachycardia and the other conditions which result from excessive pulmonary ventilation are also due to diminution of  $\text{CO}_2$ . For the solution of the problem of surgical shock this explanation of apnoea is of fundamental importance. In the extensive investigations on surgical shock performed by Crile,<sup>11</sup> it was found that in 90 per cent of the subjects death was due to respiratory failure. "In many instances the heart was beating strongly and blood pressure was fair at the time respiration failed." These observations alone would go far to show that diminution of  $\text{CO}_2$  is a factor in surgical shock, were it not for the fact that the literature of respiration presents with the weight of many authorities two other explanations of the regulation of respiration and the causation of apnoea.

<sup>7</sup> FREDERICQ : Archives de biologie, 1901, xvii, p. 563.

<sup>8</sup> MOSSO : Archives italiennes de biologie, 1903, xl, pp. 19 *et seq.*

<sup>9</sup> WEIL : Archiv für experimentelle Pathologie und Pharmakologie, 1906, liv, p. 285.

<sup>10</sup> HALDANE and PRIESTLEY : *Loc. cit.*

<sup>11</sup> CRILE : Surgical shock, 1899, p. 143.

One of these explanations is that respiration is essentially a reflex function, and that the discharges from the respiratory centre are the reaction to the augmentor and inhibitory influences of afferent nerves, and especially of the pulmonary fibres of the vagi. The standing of this explanation at the present day is estimated by Boruttau,<sup>12</sup> in the statement that the last supporter of "the purely reflex nature of the respiratory movements has taken his views to the grave with himself." It is reasonable, therefore, to reject the idea that the shallow respiration consequent on vigorous and prolonged artificial respiration is due to a lasting inhibition of the respiratory centre by stimulation through the vagi. It is reasonable also to reject the idea that the failure of respiration in shock following the hyperpnoea of pain indicates exhaustion of the respiratory centres by the stimulation of afferent nerves. Finally, by the same logic applied to closely similar facts, it is reasonable to reject the idea that the disturbances of the circulation in shock are the expression of the state into which the vaso-motor centre is thrown by the stimuli reaching it through afferent channels. Porter has in fact presented data which are opposed to the hypothesis that surgical shock is an exhaustion of this centre.<sup>13</sup> Yet this hypothesis, supported by Crile,<sup>14</sup> is generally accepted at the present time.

A third explanation of the causation of the variations in respiratory, cardio-regulative, vaso-motor and other functions has behind it a greater weight of authority, although, as we believe, a much smaller amount of evidence, if interpreted in its true significance, than either of the explanations above quoted. Because oxygen is a vital necessity, and  $\text{CO}_2$  only a waste product, it has appeared natural to many writers to infer that the oxygen tension of the blood must exert profound and extensive regulative influences.

<sup>12</sup> H. BORUTTAU: *Ergebnisse der Physiologie*, 1904, Dritter Jahrgang, ii Abteilung, p. 95. For reviews of other phases of this subject, see E. H. STARLING, *Nervous mechanism of respiration*, SCHÄFER's *Textbook of physiology*, 1900, ii, p. 283; R. MAGNUS: *Pharmakologie der Atemmechanik*, *Ergebnisse der Physiologie*, 1902, Erste Jahrgang, ii Abteilung, p. 414; R. DU BOIS-REYMOND, *Mechanik der Atmung*, same volume, p. 378; C. BOHR, *Blutgase und respiratorischer Gasaustausch*, NAGEL's *Handbuch der Physiologie des Menschen*, 1905, i, p. 54; L. FREDERICQ, on Apnoea, and RICHTER on Asphyxia, *Dictionnaire de physiologie* par CHARLES RICHTER, 1895, i, pp. 630 and 728.

<sup>13</sup> W. T. PORTER: *This journal*, 1907, xx, pp. 399 and 500.

<sup>14</sup> CRILE: *KEEN'S Surgery*, 1906, i, p. 922.

Rosenthal<sup>15</sup> and his followers attempted to explain apnoea and hyperpnoea on the ground respectively of excess and of deficiency of oxygen. Because a flame burns the brighter the richer the atmosphere is in oxygen, it appears natural to infer that the vital combustions of the body must be increased in vigor by an increase in the supply of oxygen to the lungs. That such is not the case, however, but that the rate of oxygen consumption by the animal body is independent of an excess in the supply was proved conclusively by Pflüger more than thirty years ago, and has since been verified by many investigators.<sup>16</sup>

In the recent literature the discussion of this subject has turned largely on the extensive investigations of the phenomena and causes of mountain-sickness.<sup>17</sup> Throughout the literature of this disorder, excepting only in the writings of Mosso and his adherents, its symptoms have been undoubtingly explained as due to lack of oxygen. The liberal use of oxygen in clinical medicine indicates the current opinion on this subject among physicians. Surgeons profess their faith that lack of oxygen occurs and is a cause of diminished functional activity by administering this gas to cases in shock.

Kraus in an exhaustive review of the clinical literature of this subject has expressed an opinion adverse to the value of oxygentherapy.<sup>18</sup>

Durig (*Loc. cit.* p. 363) makes the pregnant suggestion that the beneficial effects of oxygen inhalation are in great part due to the considerable quantity of CO<sub>2</sub> which the oxygen employed in the clinic usually contains. We would also call attention to the fact that the mask by which the gas is administered increases the dead-space of the respiratory tract, and thus causes the patient to some extent to re-breathe his expired air.

<sup>15</sup> J. ROSENTHAL: HERMANN'S Handbuch der Physiologie, 1882, iv, ii Theil, p. 264; Archiv für Physiologie, 1902, p. 167, and 1902 Supplement, p. 278. For a refutation of ROSENTHAL'S argument see the paper (from the laboratory of ZUNTZ) by A. DURIG: Archiv für Physiologie, 1903, Supplement, p. 209.

<sup>16</sup> PFLÜGER: Archiv für die gesammte Physiologie, i, pp. 61, 274, and 686; ii, p. 156; x, p. 251; xiv, p. 1; xix, p. 244. See also VOIT: Zeitschrift für Biologie, 1907, xlix, p. 1.

<sup>17</sup> For reviews of this literature, see O. COHNHEIM, Physiologie des Alpinismus, Ergebnisse der Physiologie, 1903, ii, 1, p. 612; ZUNTZ, LOEWY, MÜLLER, and CASPARI; Hohenklime und Bergwanderungen, 1906, chapters xi, xii, xviii, and xix; and Recent advances in physiology and bio-chemistry, 1906, edited by HILL, chapters viii and xv.

<sup>18</sup> KRAUS: Zeitschrift für klinische Medicin, xxii, p. 449.



Among physiologists the vaso-constriction and slow heart-rate of asphyxia have generally been regarded as the effects of lack of oxygen.<sup>19</sup> Verworn<sup>20</sup> in particular reached the conclusion that lack of oxygen is the primary cause of the vagus pulse in dyspnoea, and that in this respect the increase of CO<sub>2</sub> in the blood is of very minor, if of any, significance. He supported this conclusion by the statement, which if it were correct would prove his conclusions, that "from the investigations of Rosenthal and others it is to-day well established that lack of oxygen is the primary stimulus to the respiratory centre in dyspnoea, while the increased CO<sub>2</sub> as an exciting influence is of very secondary importance." It appears to us on the contrary that the literature bearing on the question affords overwhelming evidence that oxygen, not merely in respect to these functions but also in its general relations to protoplasmic activity,<sup>21</sup> exerts a relatively low potency as a hormone.

Recent investigations<sup>22</sup> have tended to show that the excitement of the vaso-motor centre in asphyxia is caused rather by the excess of CO<sub>2</sub> in the blood than by lack of oxygen. Thus the effects of a subnormal pulmonary ventilation and the consequent venous condition of the arterial blood upon the respiratory, cardio-regulative, and vaso-motor centres are all principally due to the increase of CO<sub>2</sub> rather than to diminution of oxygen. It is probable that many symptoms which physicians have regarded as due to lack of oxygen are really caused by alterations in the conditions and mechanism on which the maintenance of the normal tensions and contents of CO<sub>2</sub> in the blood, tissues, and nerve centres depend. For the needs of the body in respect to oxygen the regulation of respiration by CO<sub>2</sub> maintains a broad margin of safety.

On the effects of a reduction of the CO<sub>2</sub> tension of the blood below the normal, the most important contributions have been made by Mosso and his co-workers. In a large number of papers they have presented evidence to show that under reduced barometric pressure the CO<sub>2</sub> content of the blood is diminished, and that this

<sup>19</sup> For a review of the literature bearing on this and related questions, see R. TIGERSTEDT: *Ergebnisse der Physiologie*, 1903, ii, 2, pp. 567-571.

<sup>20</sup> M. VERWORN: *Archiv für Physiologie*, 1903, p. 65. For the views of VERWORN on the relations of oxygen and of CO<sub>2</sub> to nervous activity on his "Biogen-Hypothesis," see *Archiv für Physiologie*, 1900, Supplement, p. 152.

<sup>21</sup> Cf. J. LOEB: *Studies in general physiology*, 1905, i, p. 414.

<sup>22</sup> Cf. W. M. BAYLISS, *Die Innervation der Gefässe, Ergebnisse der Physiologie*, 1906, Fünfter Jahrgang, i Abteilung, p. 345.



diminution is one of the causes of the disturbances of function in mountain-sickness. For this condition Mosso has suggested the convenient term "acapnia" (from *καπνός*, smoke, — literally smokelessness). Mosso and Marro<sup>23</sup> have shown by blood-gas analyses that when animals are transported up to the physiological laboratory on Monte Rosa or are placed in the low pressure chamber in the laboratory in Turin, the CO<sub>2</sub> content of the arterial blood is reduced. In experiments on monkeys in this chamber, even when pure oxygen was supplied in such quantities as to eliminate the possibility of lack of oxygen playing any part in the reactions, a lowering of the barometric pressure induced muscular weakness, sleepiness, and at times almost vomiting. The observations of Mosso<sup>24</sup> and of Aggazzotti<sup>25</sup> on men, monkeys, dogs, and rabbits show that under reduced barometric pressure, when the subject is at rest, respiration is markedly diminished in amplitude while accelerated in rate. These observations may be taken on the one hand as indicating the reaction of the respiratory centre to the lower tension of CO<sub>2</sub>, and on the other as an adjustment which tends to prevent its further loss.

Considering the large number of investigators who have studied the production of apnoea by excessive artificial respiration, it is surprising that the fact is not to-day well established that acapnia immediately produces tachycardia and, if long continued, always results in shock. We certainly are not the first to observe the production of shock in this way. Thirty-four years ago Ewald, working in Pflüger's laboratory, noted that the arterial pressure of a dog which for twenty minutes was subjected to vigorous artificial respiration fell from 154 to 65 mm. of mercury. The fall was due to a very great diminution in the output of the heart, — down to 25 or 30 per cent of that during normal respiration. Pflüger showed at the same time that the oxygen consumption of the body was not altered by the ventilation. Ewald's analyses showed that the oxygen content of the arterial blood, if altered at all, was slightly increased.<sup>26</sup> The oxygen content of the venous

<sup>23</sup> Mosso and MARRO: *Archives italiennes de biologie*, 1903, xxxix, pp. 387, 395, and 402; also 1904, xli, p. 357.

<sup>24</sup> Mosso: *Archives italiennes de biologie*, 1904, xli, pp. 384 and 397; also 1904, xlii, p. 23; also 1905, xliii, pp. 81, 209, 341, 355, and 467.

<sup>25</sup> A. AGGAZZOTTI: *Archives italiennes de biologie*, 1904, xlii, pp. 14, 43, and 53; also 1905, xliv, pp. 39, 137, 150, and 343.

<sup>26</sup> A. EWALD: *Archiv für die gesammte Physiologie*, 1873, vii, p. 580.

blood was greatly diminished. In one experiment, after thirty-five minutes' ventilation, the oxygen content of the venous blood had fallen from 11.7 to 4.6 volumes per cent. The oxygen content of simultaneous samples of arterial blood was 17.3 and 17.9. The cause of the diminution in the blood stream thus indicated lay, as Ewald concluded, either in an alteration of the innervation of the blood vessels and heart, or in a venous congestion (due to the increase of resistance in the lungs by the ventilation), or in both conditions. In the experiment from which these analyses are quoted the extent of the arterial acapnia is shown by the fact that the  $\text{CO}_2$  content fell from 33.4 to 17.5. The venous acapnia in this case was relatively slight, — the figures showing 36.5 volumes per cent of  $\text{CO}_2$  before and 33.9 after thirty-five minutes' ventilation. In other experiments figures as low as 15 to 18 per cent  $\text{CO}_2$  in the venous blood were repeatedly obtained after ventilations of an hour or more. Since the tensions of the gases in the venous blood must be very nearly the same as the tensions in the tissues from which the blood flows, — just as the tensions in the arterial blood are nearly the same as those in the alveolar air of the lungs, — a very considerable washing out of  $\text{CO}_2$  from the tissues as the result of the ventilation is indicated by these figures.

In experiments similar to those of Ewald, Mosso<sup>27</sup> has likewise noted acapnia, apnoea, tachycardia, and fall of arterial pressure. He has also observed in man a lowering of blood pressure and quickening of the heart-rate coincident with the apnoea produced by forced respiration. As regards the effects of acapnia upon the circulation, the observations of Ewald, of Mosso, and of ourselves are in these respects identical. Neither Ewald nor Mosso has, so far as we can find, suggested that the condition induced by extreme acapnia is that of surgical shock. Nor, so far as we can discover, has any one prior to ourselves shown that after the opening of the thorax the heart-rate is completely controllable by regulation of the  $\text{CO}_2$  content of the blood.

Many writers have commented upon the similarity of surgical shock and mountain-sickness. Wetherill<sup>28</sup> and Powers<sup>29</sup> have observed that in Denver (altitude 5260 feet) persons who have

<sup>27</sup> MOSO: *Archives italiennes de biologie*, 1903, xl, pp. 22 *et seq.*, 1904, xli, p. 193, and 1905, xliii, p. 341.

<sup>28</sup> WETHERILL: *Annals of surgery*, 1897, xxv, p. 430.

<sup>29</sup> POWERS: *Annals of surgery*, 1897, xxvi, p. 297.

recently come from sea-level are in surgical operations peculiarly liable to shock.

Comparing the condition of the nervous system in shock with the condition induced by experimental acapnia, we find striking similarities. Rosenthal<sup>30</sup> recognized that many of the nerve centres are brought into a condition of diminished tonus and diminished irritability by the alteration in the blood gases during and after excessive artificial respiration. Kronecker and Markwald<sup>31</sup> found that under such circumstances the respiratory centre is irresponsive to direct electrical stimulation. Leube<sup>32</sup> found that in rabbits which had received a dose of strychnine sufficient to kill under ordinary conditions convulsions were prevented, and the animals recovered when a somewhat excessive rate of artificial respiration was maintained. Paul Bert noted that in rapid balloon ascensions there occurred first a period of mental excitement, followed by a period of somnolence, exhaustion and disturbances of sight and hearing. Aggazzotti<sup>33</sup> has shown on men and animals that lowered barometric pressure diminishes reflex irritability, the acuteness of hearing, and probably that of the other senses also. He likewise observed muscular weakness. Conversely Lee<sup>34</sup> has recently shown that the increase of functional activity expressed in the phenomenon of the Treppe occurs in the muscles of mammals when the tension of CO<sub>2</sub> in the blood and tissues is increased by asphyxia.

Similar relations between the tension of CO<sub>2</sub> in the tissues and their functional activity are observable in respect to the motility of the alimentary canal. It is well known that mere opening of the abdomen abolishes the peristalsis of the stomach and greatly reduces that of the intestines.<sup>35</sup> Nor is motility completely restored for a considerable period after the abdomen is reclosed. The stand-

<sup>30</sup> ROSENTHAL: HERMANN'S Handbuch der Physiologie, 1882, iv, ii Theil, p. 278.

<sup>31</sup> KRONECKER and MARKWALD: Archiv für Physiologie, 1879, p. 594.

<sup>32</sup> W. LEUBE: Archiv für Anatomie und Physiologie, 1867, p. 629; also USPENSKY: Same journal, 1868, pp. 401 and 522; also ROSENTHAL: Compt. rend., 1867, lxiv, p. 1142.

<sup>33</sup> AGGAZZOTTI: Archives italiennes de biologie, 1904, xli, p. 69; also MOSSO, 1904, xli, p. 387. Also ZUNTZ, LEOWY, MÜLLER, and CASPARI: *Loc. cit.*, pp. 420-427, 450.

<sup>34</sup> F. S. LEE: This journal, 1907, xviii, 267.

<sup>35</sup> Cf. MELTZER and AUER: This journal, 1907, xx, p. 280.

still is not due to drying, for it occurs in a bath of saline, as shown by the observations of Johannes Müller, of Pal, and recently of Auer.<sup>36</sup> On the contrary, if the trachea be occluded the exposed intestines exhibit for a brief period vigorous peristalsis. Strangely enough it appears to have occurred to no one to inquire whether (as we find to be the case) the exhalation of CO<sub>2</sub> from the moist peritoneum and the hyperpnoea induced by the operation are the causes of the cessation of peristalsis after laparotomy.

The human skin is very slightly pervious to CO<sub>2</sub>.<sup>37</sup> So long as the skin is unbroken and there is no sensible perspiration, the tissues are protected from loss of CO<sub>2</sub> except by way of the circulation. The minimum tension of CO<sub>2</sub> in any tissue can never be less than its tension in the arterial blood. This tension in turn is determined by the partial pressure of CO<sub>2</sub> in the alveolar air of the lungs. It is accordingly interesting to note that the respiratory tract of all the higher animals includes a large dead-space, — having in man a volume equal to 30 per cent of an ordinary inspiration, — which can scarcely be regarded as useful in respect to the oxygen supply of the body, but is an important factor in the maintenance of the normal high tension (5.5 per cent of an atmosphere) of CO<sub>2</sub> in the alveolar air. Paul Bert<sup>38</sup> found that the CO<sub>2</sub> content of the arterial blood was diminished after tracheotomy. Thus:

	Dog A		Dog B	
	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>
Before tracheotomy . .	15.1	40.8	16.0	41.5
After tracheotomy . .	20.3	24.0	23.4	15.2

If the CO<sub>2</sub> content of the blood supplied to the nervous system exerts under the normal conditions of life regulative influences of the potency which our investigations indicate, it would appear that could an individual acquire the ability voluntarily to increase or diminish the rate of pulmonary ventilation marked alterations in the condition of the nervous system might be expected to result. In fact breathing exercises have in recent years been successfully employed as part of the treatment for neurasthenia in some sanitariums. Among a number of the semi-religious, semi-philosophical cults and sects which have gained adherents in America in recent

<sup>36</sup> AUER: This Journal, 1907, xviii, p. 347.

<sup>37</sup> SCHIERBECK: Archiv für Physiologie, 1893, p. 123.

<sup>38</sup> P. BERT: La Pression barometrique.

years, and which have in one form or another held up as the chief object of their practices the attainment and maintenance of physical health and the control of the vegetative functions of the body by the mind, breathing exercises have been extensively developed. Noteworthy are the elaborate breathing exercises of the Yoga or Vedanta Philosophy.<sup>39</sup> For more than two thousand years these exercises have been practiced in India as the means through which, as claimed, the subject can gain control over the heart-rate and other functions, can render himself insensitive to pain, or even unconscious, and can induce mental exaltation and hallucinations. In some of the older works on surgery it was directed that prior to a minor operation the subject should for a few minutes perform forced respiration. The relations of pain to respiration, and of respiration to pain, are as yet little understood.

Finally the question arises, Do variations in the  $\text{CO}_2$  content of the arterial blood play any considerable part in conditioning the variations in the heart-rate which in a normal man occur with every bodily movement, with every change of position, and with every emotion? The investigations of Haldane and Priestley already quoted make it altogether probable that under nearly all normal conditions the variations in the  $\text{CO}_2$  tension in the arterial blood are very slight. The threshold exciting value of the respiratory centre in respect both to increase and to diminution in the tension of  $\text{CO}_2$  is so low in comparison with the other centres, such as the cardio-inhibitory, that the above question can be answered quite clearly in the negative. Hypo- and hyper-capnia are abnormal conditions. Moreover, the investigations of Fredericq<sup>40</sup> on the relations of the heart-rate to the activity of the respiratory centre have demonstrated the intimate sympathy between this centre and those controlling the heart-rate. Thus respiratory excitement (or indeed excitement in any part of the nervous system) always exerts a cardio-accelerative influence. Voluntary holding of the breath by a man, or breathing through a large dead-space, or asphyxiation on an animal causes primarily not a slowing but an acceleration of the heart-rate because of the respiratory excitement. The in-

<sup>39</sup> For an interesting account of these practices and their results, see the article by Professor WM. JAMES, *Science*, 1907, xxv, pp. 326-328.

<sup>40</sup> FREDERICQ: *Archives de biologie*, 1882, iii, p. 75. For the extensive literature of this subject, see TIGERSTEDT, *Lehrbuch des Kreislaufes*, 1893, p. 453; also TIGERSTEDT, *Ergebnisse der Physiologie*, *loc. cit.*

stant this respiratory excitement ceases the heart-rate becomes extremely slow because (as the work of Verworn<sup>41</sup> and others has shown) of the asphyxial condition of the blood. Vagus-tonus<sup>42</sup> is generally regarded as the principal factor in determining the heart-rate. It appears therefore that an asphyxial (or hypercapnial) condition of the blood supply to the spinal bulb exerts a stimulating influence upon both the respiratory and the cardio-inhibitory centres. Experiments of Mosso,<sup>43</sup> in which the heart-rate even after vagus section was accelerated by excessive artificial respiration, make it probable also that acapnia stimulates or that CO<sub>2</sub> exerts an inhibiting influence upon the cardio-accelerator mechanism.

Although variations in the CO<sub>2</sub> content of the blood are therefore not factors in the momentarily occurring normal variations in the heart-rate, the heart-rate of every individual when at rest is, we believe, principally determined by the CO<sub>2</sub> content of the blood. Slight alterations in the sensitivity of the respiratory centres and the consequent alterations in the tension of CO<sub>2</sub> in the blood would afford a sufficient cause for the variations in the pulse rate which occur in many abnormal conditions.

Hypo-capnia has in fact been found to occur in many forms of fever both in men and animals,<sup>44</sup> in diabetic coma,<sup>45</sup> in the condition consequent on violent muscular exercise,<sup>46</sup> and in that form of shock which results from the intravenous injection of albumose or "peptone."<sup>47</sup> In all of these conditions, as well as in mountain-sickness, excessive artificial respiration, and surgical shock, there occur at one stage or another and in varying order hyperpnoea, followed by shallow respiration or apnoea, tachycardia, venous congestion and fall of arterial pressure, muscular weakness, suppression of reflexes, disturbances of consciousness, and disturbances of the motor functions of the alimentary canal.

<sup>41</sup> VERWORN: *Loc. cit.*

<sup>42</sup> For literature, see F. B. HOFMANN, in NAGEL'S *Handbuch der Physiologie des Menschen*, 1905, i, Erste Hälfte, p. 276.

<sup>43</sup> MOSO: *Archives italiennes de biologie*, 1904, xli, p. 200.

<sup>44</sup> For a review and bibliography of this subject, see L. KREHL, *Pathologische Physiologie*, third edition, 1904, pp. 459 and 475.

<sup>45</sup> BEDDARD, PEMBREY, and SPRIGGS, *Journal of physiology*, 1904, xxxi, p. xlv.

<sup>46</sup> HILL and FLACK, *Journal of physiology*, 1907, xxxvi, p. xi.

<sup>47</sup> LAHOUSSE: *Archiv für Physiologie*, 1889, p. 77.

CARDIAC TETANUS.<sup>48</sup>

The object for which our earlier experiments were planned was to record the volume curve of the ventricles of the heart. Twenty-five dogs were used.

The animals received subcutaneously a moderate dose of morphine and were then anesthetized with ether. The thorax was opened by an incision directly down through the skin and through all of the costal cartilages at their junctions with the ribs, so that the sternum and costal cartilages with their adherent musculature and skin formed a lid hinging upon the clavicles. A single ligature was then placed about the internal mammary arteries and veins near the origin of the arteries. In none of the experiments to be discussed in this paper was there any considerable hæmorrhage. The pericardial sack was opened and rolled back from the heart. A cardiometer (described in the previous paper already referred to) was slipped on over the ventricles so that their volume changes were recorded by a large tambour connected with the interior of the cardiometer by a rubber tube. Blood pressure was recorded by a Hürthle manometer connected either with the carotid or with a sound inserted into the left ventricle.

Owing to the fact that the apparatus for artificial respiration happened at the time to be out of order, air was supplied to the lungs by means of a large hand bellows connected directly with a cannula tied into the trachea. The animals were thus subjected to a ventilation which, as later experiments have shown, was in excess of their needs. We were at that time unaware of the evil effects of excessive pulmonary ventilation. When it was seen that the blood pressure was falling in spite of (or because of) a tremendous heart-rate, we supposed that the respiration must be insufficient. Accordingly the escape vent of the tracheal cannula was opened wide, and the lungs were filled as fully and at as rapid a rate as possible. In fact the arrangement of the respiration apparatus was such as not only to fill the lungs to their maximum capacity with each down-stroke of the bellows, but also to exert with each up-stroke a slight suction on the lungs, and thus to accelerate their collapse. The alveolar air must thus have been kept nearly at the same composition as that of the laboratory, while normally it contains some 5 or 6 per cent of CO<sub>2</sub>.

<sup>48</sup> For the literature bearing on this subject and for an experimental demonstration that the mammalian heart is capable of tetanus, see DANILEWSKY, *Archiv für die gesammte Physiologie*, 1905, cix, p. 596.



Under such treatment it twice happened that the subject of the experiment was dead within half an hour after the thorax had been opened and artificial respiration begun. The cause of death was the cessation of the pumping action of the heart. The ventricles became so contracted, because of the great rapidity of beat and extreme Treppe, that the ventricular chambers were almost obliterated. Immediately after death the cardiometer was removed, and it was found that although the auricles were still beating, the ventricles were hard and incompressible. To the eye and finger the muscle appeared to be in a complete tetanus. A few minutes later the right ventricle relaxed and was responsive to mechanical stimulation.

Since the thorax was open it is improbable that mechanical obstruction to the pulmonary circulation played any considerable part in the results. Doubts on this point are further negated by the results of later experiments.

#### ACAPNIAL SHOCK UNDER ARTIFICIAL RESPIRATION.

In those experiments in which the artificial respiration was administered less vigorously, although still in excess, the progress of events was much less rapid. Tachycardia developed to a degree sufficient to cause a marked but not an immediately fatal fall of arterial pressure. The animals then passed into a condition of shock; and death, which in these cases occurred in from one to three hours after the opening of the thorax, was due not to cardiac tetanus, but to failure of vascular tonus. Indeed, in later experiments it was found very difficult to induce cardiac failure through extreme tachycardia except in small animals, in which the thorax was widely opened and the pulmonary ventilation extremely rapid.

In the larger animals there developed quite uniformly a stage in which the up-strokes of the volume curve of the heart (indicating the rate of filling of the ventricles during diastole) were much less abrupt than when the animals were fresh. This retardation of the diastolic filling was especially noticeable at intervals when, coincident with a less vigorous working of the bellows, the rapidity of the heart-rate was reduced. At such times the heart did not recover the amplitude of beat normal for the slower rate. The volume of blood pumped out into the arterial system by the heart was therefore greatly reduced. The alteration in the form of the

volume curve was similar to that exhibited by a striated muscle in the slow relaxations of the early stages of fatigue. This similarity, however, was only superficial. The heart was not fatigued. When saline was infused into a vein the abruptness of the up-strokes of the volume-curve immediately reappeared. During the time that the beneficial effects of the infusion lasted the normal amplitude of beat was regained, and arterial pressure was restored to a normal or nearly normal level. These observations indicated clearly that, as one of the results of excessive pulmonary ventilation, the tonus of the venous system was reduced to a point at which a stasis of the blood in the veins occurred. Thus the venous supply to the right heart was reduced. Finally the venous supply failed almost completely; the pumping action of the heart was abolished; and the arterial circulation ceased.

The conditions observed in these experiments appear to be identical with those which clinical and experimental observations have established as the cause of the failure of the circulation in surgical shock.<sup>49</sup> As regards the state of the respiratory centre, a prolonged apnoeic pause was noted invariably when the artificial respiration was suddenly discontinued in the earlier stages. In the later stages we have repeatedly seen the apnoeic pause prolonged until the heart ceased to beat (probably from lack of oxygen), without the slightest respiratory effort on the part of the animal. Equally marked were the effects of the excessive pulmonary ventilation upon other centres in the nervous system. The animals were usually only partially morphinized. Under moderate artificial respiration a constant administration of anæsthetic (usually ether) was necessary. Under excessive artificial respiration they usually appeared almost completely comatose. In this condition no anæsthetic was needed. This was the case even during the earlier stages, while arterial pressure was at a normal level or even above the normal. Even in the earlier stages vigorous stimulation of an afferent nerve (pinching, or electrical stimulation of the sciatic nerve, burning the foot, etc.) often failed to elicit a rise of arterial pressure. In the later stages such stimulations were invariably

<sup>49</sup> CRILE: An experimental research into surgical shock, 1899; CRILE: Blood pressure in surgery, 1903; HOWELL: Contributions to medical research dedicated to V. C. VAUGHAN, 1903, p. 51; DAWSON: Journal of experimental medicine, 1905, vii, p. 1. For reviews of the experimental and clinical literature, see MORMERY, Lancet, 1905, i, pp. 696, 776, and 846; also CRILE, on Surgical physiology and on shock and collapse, in KEEN'S Surgery, 1906, i, pp. 79 and 922.

wholly ineffective upon arterial pressure, even when sufficiently strong to elicit respiratory movements out of profound apnoea.

With the exception of complete tetanus, all of the variations in the circulation thus far discussed, both the genesis of cardiac tetanus, with its consequent fall of arterial pressure, and the be-

TABLE I.  
TO ACCOMPANY FIGURE 1.

Time.	Arterial blood gases.		Heart-rate per minute.	Amplitude of beat.	Output of left ventricle per minute.	Arterial pressure.	Remarks.
	O <sub>2</sub> .	CO <sub>2</sub> .					
	vols.	per cent.		c.c.	c.c.	mm. of Hg.	
11.30	22.7	37.3	115	..	....	110	
12.10	22.7	35.5	130	..	....	110	
12.45	6.4	39.1	75	38	1425	125	
1.00	....	....	80	23	920	60	Excessive ventilation.
1.10	....	....	125	15	937	50	Excessive ventilation.
1.15	19.1	7.2	230	5	575	60	Excessive ventilation.
1.25	....	....	120	9	540	35	Apnoea.
1.50	....	....	95	8	380	15	Profound shock.
2.15	....	....	180	12	1080	40	Saline infu- sion.
2.30	....	....	180	20	1800	80	
3.00	....	....	180	11	990	50	Relapse.
3.10	....	....	60	13	390	30	Profound shock.
3.15	....	....	50	15	375	20	Profound shock.
3.30	....	....	110	7	385	15	Death.

havior of the heart after loss of vascular tonus, with the consequent diminution in the blood stream, are illustrated in the record (Fig. 1) taken from one of our later experiments. In connection with this experiment the O<sub>2</sub> and CO<sub>2</sub> content of four samples of blood (10 c.c.) drawn from the femoral artery at various rates of heart-beat were determined by means of the blood gas-pump of Leonard Hill.<sup>50</sup> In the table are given the results of these analyses calculated to 0° and 760 mm. of mercury, together with

<sup>50</sup> L. HILL: *Journal of physiology*, 1895, xvii, p. 353.

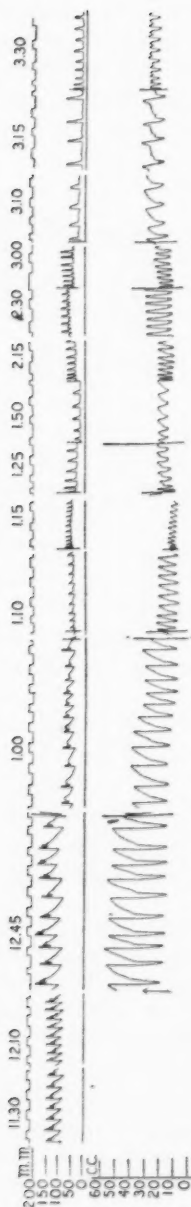


FIGURE 1. — Experiment of July 16, 1906. Dog of 14 kilos. Morphine and ether. Time record in 0.5 second. Arterial pressure recorded from carotid by Hurtle manometer; record graduated in mm. of mercury. Volume curve of ventricles recorded by large tambour connected with plethysmograph on the heart; record graduated in c.c. so that one-half the indicated amplitude of each down-stroke represents the volume of the systolic discharge of the left ventricle. The thorax was opened at 12.15. Excessive artificial respiration was administered for 20 minutes, and the heart-rate was then slowed by lessened artificial respiration until the subject made spontaneous respiratory movements. The cardiometer was placed on the heart (indicated by an arrow) and the first volume curve taken at 12.45. The pulse at this time shows a slight respiratory variation. The artificial respiration was then administered with steadily increasing vigor up to a maximum rate of ventilation at 1.15. The heart-rate increased correspondingly from 80 per minute at 12.45 up to 230 at 1.15. The amplitude of beat diminished correspondingly from 38 c.c. down to 5 c.c. (for both ventricles), and the output of the left ventricle diminished therefore from 1425 c.c. per minute down to 575 c.c. Further increase in the heart-rate to about 300 beats per minute would have brought the heart so nearly into tetanus that its pumping action would have been abolished. Instead of maintaining the excessive ventilation the working of the bellows was diminished at 1.20. The subject passed into shock. At 1.50 the heart-rate was 95, yet owing to the slow filling of the heart in diastole the amplitude was only 8 c.c. and the output per minute of the left ventricle only 380 c.c. Arterial pressure accordingly had fallen to only 15 mm., and the animal was at the point of death. From 2.10 to 2.25 300 c.c. of saline were infused into the femoral vein. The amplitude of the volume curve at 2.30 was thus restored to normal (20 c.c.) for the coincident rate of beat (180 per minute) and the output of the left ventricle rose to 1800 c.c. Arterial pressure was raised thereby to 80 mm. By 3.10 the beneficial effect of the infusion had so far disappeared and the heart was filled so incompletely during diastole that the output of the left ventricle per minute was only 390 c.c. By 3.20 vascular tonus, both arterial and venous was practically abolished. Although so long as artificial respiration was maintained the heart continued to beat feebly, the animal was practically dead at 3.25. See Table I on p. 145.

the heart-rates counted from the pulse curve, and the volume of the blood stream as calculated by multiplying the heart-rate and amplitude of the volume curve, and dividing their product by two, so as to give the output of the left ventricle per minute.

#### ARTIFICIAL REGULATION OF THE HEART-RATE.

After long uncertainty and many experimental failures, the idea forced itself upon us that in an animal with open thorax, under artificial respiration, and in the absence of any spontaneous respiratory movement, the heart-rate is almost wholly determined by the rate of the pulmonary ventilation. The respiration apparatus shown in Fig. 2 was devised as a simple means of obtaining any

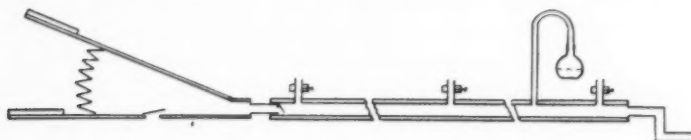


FIGURE 2.—In Figure 2 is shown the form of respiration apparatus which, out of several modifications, proved the most convenient for the regulation of the heart-rate. The large hand bellows was fitted with valves both at the inlet and outlet. To the outlet of the bellows was attached air tight a tube 200 cm. long and from 1.5 to 4.0 cm. interior diameter according to the size of the animal. For large animals two Liebig condenser jackets connected end to end make a convenient tube. The other end of this tube was connected air tight with the cannula tied into the trachea. Near both ends and at the middle of the tube were adjustable escape vents. Near the tracheal end of the tube was another opening to which a bottle containing ether was connected by a short piece of rubber tubing. When the bottle was inverted a few drops of anæsthetic fell into the tube.

desired rate of pulmonary ventilation simultaneously with a sufficient movement of the lungs to inhibit through vagus apnoea all spontaneous respiratory effort by the subject. The necessity of producing a considerable movement of the lungs simultaneously with a very slight ventilation with fresh air, when very slow heart-rates are to be induced, arises from the fact that otherwise the venous condition of the arterial blood under slight aeration induces a hyperpnoea which sympathetically accelerates the heart-rate. By means of this apparatus the heart-rate can be regulated to any desired rhythm from below 50 up to more than 200 beats per minute. In our hands it has never induced cardiac tetanus even when worked to the utmost. In all experiments in which excessive

ventilation was maintained for any considerable period shock developed. On the other hand shock never developed, even when the period of observation lasted for the greater part of a day, in those experiments in which excessive ventilation and tachycardia were maintained for only a few minutes at a time, and in which the heart-rate was then slowed down to a normal rhythm by diminished ventilation, — providing sufficient care was exercised not to carry out the latter process so rapidly as seriously to impair the oxygen supply.

When a normal slow heart-rate was desired, the middle vent was opened slightly, and the bellows was worked at about twenty strokes per minute of moderate amplitude. When a gradually accelerating heart-rate was desired, the vent near the trachea was opened and the middle vent closed without changing the rate and depth of respiration. When tachycardia was desired, the tracheal vent was opened widely, and the bellows was worked so as to give as full and as rapid a respiration as possible. Under these conditions a very thorough pulmonary ventilation was effected. In dogs of moderate size the heart-rate within two to four minutes could be accelerated from 50 or 60 up to 180 or 200.

When a restoration of the slow heart-rate was desired, the middle vent alone was opened, and this vent only slightly. At first barely sufficient respiration was given to keep the lungs distended. The exhalation of  $\text{CO}_2$  was thus reduced, and a condition afforded in which the blood and tissues gradually recuperated their supply. During this procedure arterial pressure and the amplitude of the heart-beat (as shown either in the volume curve or in the pulse curve) were watched with the utmost care. A diminution in either of these functions was taken as an indication that the oxygen supply was being reduced beyond the point of safety. Several times in our earlier experiments it happened that during this procedure, after an extreme and unusually prolonged excessive ventilation, the amplitude of the heart-beat narrowed, arterial pressure fell, and the subject came to the point of death without the slightest spontaneous respiratory effort, without any marked preliminary slowing of the heart-rate, and without a rise of arterial pressure. From these observations it appears that lack of oxygen is not the cause of the symptoms characteristic of asphyxia. Lack of oxygen apart from excess of  $\text{CO}_2$  is not a stimulus. It merely paralyzes.

When care was taken not to diminish the ventilation beyond the

point at which the arterial blood was only slightly darkened in color, the process of restoring the  $\text{CO}_2$  content of the blood and regaining a slow heart-rate required a half hour or more according to the extent of the acapnia previously induced. From a normal rate the heart could for a few minutes at a time be slowed down to rates of only 20 to 30 beats per minute. This object was accomplished by opening only the vent next the bellows. In this case nearly all of the fresh air escaped even when the bellows was worked with vigor. The dead air in the tube was merely moved back and forth between the tracheal end of the tube and the lungs. The pulmonary ventilation was thus reduced almost to nil, but a sufficient movement of the lungs was effected to induce vagus apnoea. Spontaneous respiratory movements sometimes occurred in spite of this inhibition. Arterial pressure was maintained in spite of the bradycardia. From these observations, coupled with those described in the preceding paragraph, it appears that the excess of  $\text{CO}_2$  in the blood is the stimulus to the respiratory, cardio-inhibitory, and vaso-motor centres which causes the symptoms characteristic of asphyxia.

Including both our earlier and later experiments on artificial respiration with open thorax, thirty dogs were used. In connection with five of the later experiments blood gas analyses were performed. The following is an example of one of these later experiments.

*Experiment of July 3, 1906.*—Dog of 22.0 kilos. Morphine and ether. Tracheotomized and thorax opened at 10.10, cardiometer placed on the ventricles, and artificial respiration maintained with the apparatus shown in Fig. 2. The graphic records obtained during the period of observation from 10.00 A. M. to 5.15 P. M. will be reproduced in a later paper. They consist of the four-fold repetition of such a record as that in Fig. 1 from 12.45 to 1.15. The data obtained are summarized in Table II, p. 150.

In this experiment an example is afforded of the prevention of shock by care in the administration of artificial respiration. The intervals of excessive ventilation were never prolonged beyond a few minutes. Seven hours after the opening of the thorax, and prior to the last interval of excessive ventilation, the animal was in such good condition that it might easily have been restored to normal life, if antiseptic precautions had been employed. Throughout the seven hours of observation the heart-rate was varied at the



will of the operator at the bellows with the ease and almost with the precision that the rate of the ticking of a clock is varied by changes in the length of its pendulum.

TABLE II.

DATA OBTAINED IN EXPERIMENT OF JULY 3, 1906. — See p. 149.

Time.	Arterial Blood Gases.		Heart-rate per minute.	Arterial Pressure.	Remarks.
	O <sub>2</sub>	CO <sub>2</sub>			
	volumes.	per cent.		mm. of Hg.	
10.00	20.1	42.6	68	95	Profound anæsthesia.
10.30	....	....	84	105	Under artificial respiration after tracheotomy and opening of thorax.
10.45	....	....	120	110	Increased ventilation.
11.15	15.1	45.0	45	65	Very little ventilation.
11.30	....	....	160	75	Increased ventilation.
12.00	....	....	84	120	Diminished ventilation.
12.07	20.9	25.8	220	80	Excessive ventilation.
1.05	19.1	35.2	96	110	Diminished ventilation.
1.10	....	....	20	75	Vagus Apnoea, no ventilation but vigorous artificial respiratory movement.
2.15	....	....	60	105	Moderate ventilation.
2.16	....	....	200	85	Excessive ventilation.
2.45	....	....	90	90	Diminished ventilation.
3.30	....	....	72	100	Diminished ventilation.
5.00	....	....	40	95	Very little ventilation.
5.15	....	....	220	60	Excessive ventilation.

#### SHOCK AND ITS PREVENTION UNDER THE SAUERBRUCH-BRAUER METHOD OF RESPIRATION.

The first clear indication of the relation of excessive pulmonary ventilation to the development of shock, and the starting-point for all of our later investigations in this field, occurred in connection with experiments in which, after the opening of the thorax, a natural respiration of compressed air was maintained. This method of respiration was originated by Sauerbruch;<sup>51</sup> and simplified by

<sup>51</sup> SAUERBRUCH: *Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie*, 1904, xiii, art. xvii.

Brauer.<sup>52</sup> At the time of its publication, three years ago, it excited a very general interest both among surgeons and experimenters. By the employment of this method in either its original or its modified form, it seemed not too much to hope that the thoracic viscera would become almost as readily accessible as a field of surgical operation as are those of the abdomen. For purposes of experimentation it appeared to conserve more nearly normal conditions after pneumo-thorax than are afforded by artificial respiration. Several American investigators who have tried the Brauer method have found, however (as they have verbally informed the writer), that the results obtained are in fact much less satisfactory than are those afforded by artificial respiration. Likewise in Germany Sauerbruch and his adherents have found the Brauer modification unsatisfactory, although they have not succeeded in proving any essential difference either theoretical or practical between the original and the modified method. Seidel has observed that under the "Überdruckverfahren" of Brauer tracheotomized animals fare much worse than those which breathe by mouth and nose into the pressure chamber.<sup>53</sup>

In our experience, on the contrary, the first trial of the Brauer method shortly after its publication was strikingly successful. When the thorax was opened, the trachea was connected with a large gasometer by a piece of rubber hose two centimetres in diameter and a metre in length. At the point where the hose connected with the gasometer the air was allowed to escape through a side tube leading to a Mueller valve adjusted to maintain the air in the system at a positive pressure of 11-13 cm. of water. The lungs were thus kept at a nearly normal distention, and the respiratory movements of the diaphragm and the thoracic walls were effective. Throughout and up to the end of the six hours of the experiment the animal maintained a deep steady respiration, high blood pressure, and normal heart-rate. Yet in addition to the extensive initial operation, a plethysmograph was placed about the ventricles of the heart, another upon the right auricular appendage, and a catheter was inserted into the left ventricle through the carotid. Three of the graphic records obtained in this experiment were published in our earlier paper.

Equally signal failures were the next three experiments. Yet

<sup>52</sup> BRAUER: *Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie*, 1904, xiii, art. xviii.

<sup>53</sup> SEIDEL: *Ibid.*, 1907, xvii, art. xxiv (complete bibliography).

the operative and experimental procedures were the same as in the first experiment, except that the apparatus supplying air was improved (as we then supposed) by removing the side tube leading to the Mueller valve from the end of the hose connected to the gasometer and inserting it at the junction of the hose with the tracheal cannula. In this manner we thought to afford a much fresher supply of air to the lungs; for if we consider that under the earlier arrangement the hose constituted merely an elongation of the trachea, it is evident that by this change the stationary air was diminished and the pulmonary ventilation increased. Under these conditions three successive animals, each a fair-sized and vigorous dog, collapsed rapidly. The heart-rate rose in the course of the first half hour to 180 or more per minute. The amplitude of the pulse and volume curves was progressively diminished. In two cases arterial pressure began to fall after two hours. The pulse slowed somewhat, but was still rapid. The amplitude of the heart-beat and pulse was not recovered. The respiration became shallow. At the end of three hours the animals were in a dying condition. In another case the animal ceased to breathe after an hour and a half, although the arterial pressure had not fallen and the pulse was about 200 per minute. Indeed, a tendency to apnoea is a constant and significant symptom accompanying tachycardia. Both symptoms are due to a subnormal  $\text{CO}_2$  content in the blood supply to the cardiac and respiratory centres.

A fifth trial of the Sauerbruch-Brauer method yielded by accident the key for the adjustment of the method to the respiratory needs of an animal. The conditions of the experiment were the same as in the three preceding. Within half an hour the animal had developed a marked tachycardia. Believing that such a failure as the experiment promised to be might as well be ended immediately, one of us turned off the stop-cock on the gasometer, to which was connected the hose leading to the tracheal cannula. The lungs were thus left distended, and the animal for a number of successive respirations repeatedly inspired and expired the same air so far as the respiratory movements were effective. To our astonishment the heart-rate immediately slowed down. The volume curve of the heart and the pulse curve regained their normal amplitude. When, however, the fresh air was turned on again by reopening the stop-cock on the gasometer, the rhythm of beat, after remaining for a few minutes normal, again increased in rapidity.

Excessive pulmonary ventilation was thus in these experiments also indicated as the cause of tachycardia and of the development

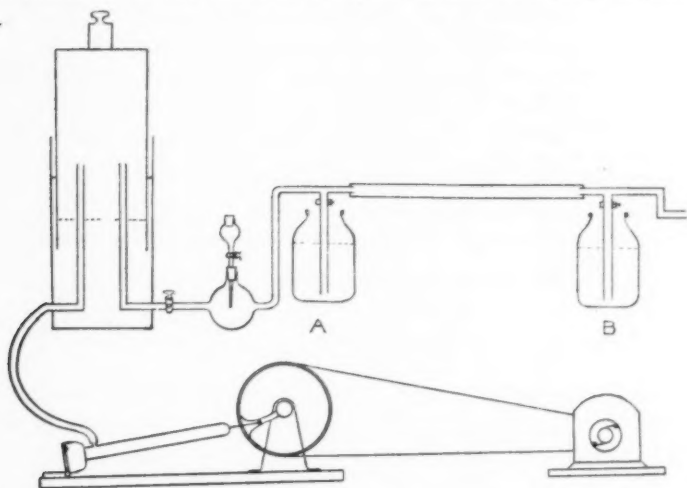


FIGURE 3. — Air was forced into the large gasometer by an automobile pump worked by an electric motor. A weight was placed on top of the gasometer sufficient to give a pressure on the air it contained of about 13 cm. of water. The air supply was largely in excess of even the extreme needs of a large animal, so that not only was the gas tank kept full but air also constantly bubbled from under its edge and escaped. The outlet tube of the gasometer opened into a flask of about a litre capacity. This flask had two other openings. Into the uppermost opening was inserted a small separatory funnel. By blowing into the funnel through a tube (not shown) the ether was forced into the flask as needed. From the third opening a piece of large rubber hose (2 cm. interior diameter and 30 cm. long) led to a T-tube.<sup>54</sup> The side opening of this T-tube was connected by a short piece of large rubber tubing (10 cm. long) with a glass tube (2 cm. interior diameter and 20 cm. long) which hung in a jar of water (*A*) so that the lower end was 13 cm. below the surface of the water. To the third opening of the T-tube was attached a piece of rubber tubing (2 to 4 cm. interior diameter and 50 to 200 cm. length according to the size of the animal). At its other end this tube connected with another T-tube similar to the first and similarly connected to a tube hanging in another jar of water (*B*). The third opening of this T-tube was connected with the tracheal cannula by as short a piece of rubber tubing as possible. Either of the Mueller valves thus formed could be shut off by pinching the rubber tubing (above *A* or *B*) which connected the side openings of the T-tubes with the glass outlet tubes hanging in the jars of water.

of shock. In the long series of experiments which followed and which form the second series in our previous paper, the heart-rate was regulated by means of the apparatus shown in Fig. 3.

<sup>54</sup> The T-tubes were obtained from a plumber. Pieces of gas pipe 5 cm. in length were screwed into their openings.

With this apparatus variations in the pulmonary ventilation were effected in a very simple manner. When a full ventilation was desired the Mueller valve near the gasometer was closed and that near the trachea opened. When a regulated ventilation was desired the former was opened and the latter nearly or completely closed. A further regulation was obtained by means of the stop-cock, which opened from the gasometer into the etherizing flask.

TABLE III.  
TO ACCOMPANY FIGURE 4.

Time.	Arterial blood gases.		Heart-rate per minute.	Amplitude of beat.	Output of left ventricle per minute.	Arterial pressure. mm. of Hg.	Remarks.
	Volumes O <sub>2</sub> .	Per cent CO <sub>2</sub> .					
3.15	17.1	41.3	60	c.c. 27-40	c.c. 1050	130	Regulated ventilation.
3.55	19.5	29.4	150	12	900	105	Full " "
4.10	...	...	90	12-28	870	125	Regulated " "
4.30	...	...	160	11-14	960	115	Full " "
5.05	...	...	70	15-33	755	130	Regulated " "
51.5	...	...	100	16	800	110	Full " "
5.35	...	...	150	11	825	70	" " "
6.15	...	...	160	8	640	40	Shock.

Even under the fullest ventilation by this method the acceleration of the heart-rate was relatively slow as compared with that attainable under artificial respiration. At the first attempt an increase in half an hour from 70 or 80 up to 150 beats per minute was the usual acceleration. After the animal had once become distinctly acapnic the change from a slow to a rapid heart-rate could be effected much more quickly. The slow pulse induced by diminished ventilation after an interval of acapnia was always characterized by an alternation of slow full beats during the respiratory pause and quicker narrower beats during inspiration.

On the question why an animal after the opening of its thorax should breathe itself into acapnia we have as yet no sufficient explanation. It appears probable that an exhalation of CO<sub>2</sub> from the pleura may occur. Even if this exhalation occurs, however, it

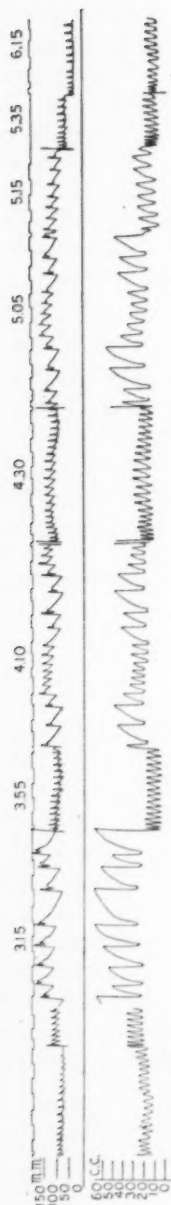


FIGURE 4. — Experiment of May 15, 1906. Dog of 15.0 kilos. Morphine and ether. The thorax was opened at 2.15, and the respiration apparatus (Figure 3) connected with the trachea. Time record in 0.75 seconds. Carotid pressure was recorded by a Hürthle manometer, and the volume curve of the heart by a tambour connected with a plethysmograph over the ventricles. Compare Table III.

does not explain the failure of the normal adjustment of pulmonary ventilation to the respiratory needs of the animal. This matter will require further investigation. The point to be emphasized here is the fact that throughout our experiments, when acapnia was altogether prevented, shock never developed even when the period of observation lasted all day.

Including both our earlier and later experiments with this method of respiration twenty-five dogs were used. In connection with three of the later experiments blood gas analyses were performed. Fig. 4 and the analytical data accompanying it afford an example of one of these later experiments.

#### SUMMARY.

1.<sup>55</sup> In dogs under artificial respiration the development of shock is dependent not upon the extent of the injuries and the intensity of the stimulations to afferent nerves, but upon the rate of pulmonary ventilation.

2. The hypothesis is presented that acapnia (diminished  $\text{CO}_2$  in the blood and tissues resulting from hyperpnoea and from exhalation of  $\text{CO}_2$  from exposed viscera) is the cause of surgical shock.

3. The literature of  $\text{CO}_2$  as a hormone is reviewed in order to show that the failure of the circulation and of the nervous system in shock and the cessation of respiration in apnoea vera must logically be referred to the same cause, — acapnia.

<sup>55</sup> The numerals refer to the sections summarized.

4. Experiments are described in which by the effects (probably upon the centres in the spinal bulb) of a sudden great diminution in the  $\text{CO}_2$  content in the arterial blood the heart-rate was increased up to the point of cardiac tetanus, and death resulted.

5. In experiments in which less extreme but still excessive artificial respiration was maintained for an hour or more a condition of typical surgical shock developed.

6. By regulating the rate of pulmonary ventilation by the method here described the heart can be adjusted to any desired rate of beat.

7. The reason for the unsatisfactory results obtained with the Sauerbruch-Brauer methods of respiration is to be found in the development of acapnia. Prevention of acapnia by the method here described prevents the development of shock.

The next three papers of this series, for which the data are already complete, will deal with the production and prevention of shock from exposure of the abdominal viscera, and from the hyperpnoea of pain and of ether excitement.



## THE PYRIMIDINE DERIVATIVES IN NUCLEIC ACID.<sup>1</sup>

By THOMAS B. OSBORNE AND F. W. HEYL.

[From the Laboratory of the Connecticut Agricultural Experiment Station,  
New Haven, Conn.]

BURIAN<sup>2</sup> has raised the question whether or not the uracil and cytosine that are obtained by severe hydrolysis of nucleic acids exist preformed in the molecule or are formed from the purines by the action of strong sulphuric acid in the presence of carbohydrates. Experiments which Burian made by heating a mixture of guanine or adenine and various carbohydrates with 30-40 per cent sulphuric acid were the basis for this question. He obtained pyrimidine derivatives, one of which resembled the isocytosine of Wheeler.

In view of the controversy which this question has raised it is important to obtain evidence which will be conclusive as to the primary or secondary origin of the pyrimidines from nucleic acid. The real question is not, *can* the pyrimidines be thus obtained from the purines, but, *are* they present as such in the molecule of nucleic acid?

This ought to be answered by direct experiments on the acid itself, and not, as has thus far been done, by experiments on mixtures of purines and carbohydrates with sulphuric acid, for the results of such experiments do not give a direct answer to the question involved.

It seemed to us that a definite conclusion could be easily reached if the purines were first removed from the nucleic acid by a mild hydrolysis and, after separating them from the solution, subjecting the remainder of the acid to the severe hydrolysis necessary for the liberation of the pyrimidines. The essential elements of such an experiment depend on obtaining positive evidence that the purines have been completely separated and on the subsequent isolation and absolute identification of the pyrimidines. The proof of the absence

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

<sup>2</sup> BURIAN: *Zeitschrift für physiologische Chemie*, 1907, li, p. 438.

of the purines is easily obtained by showing that no ammonia is formed during the severe hydrolysis, for each molecule of pyrimidine that is formed from the purines involves the production of two molecules of ammonia.

The results of the experiments that we have tried show that almost exactly ten sixteenths, or 64 per cent, of the nitrogen of tritico-nucleic acid is split off by boiling for two hours with 2 per cent sulphuric acid, and that by heating the remainder of the acid with 20 per cent sulphuric acid for two hours at  $150^{\circ}$  no ammonia whatever is formed, and that both cytosine and uracil result in notable quantity. From this we can conclude that these pyrimidines are primary decomposition products of the tritico-nucleic acid, and further that uracil is not derived from cytosine, for this also involves the production of ammonia.

We have further found that approximately three sixteenths of the nitrogen of the nucleic acid is precipitated by phosphotungstic acid and two sixteenths is not thus precipitated, thus indicating that one molecule of cytosine and one of uracil are yielded by the nucleic acid for every four atoms of phosphorus.

#### EXPERIMENTAL.

In an oil bath 20.8 gm. of tritico-nucleic acid were boiled with 150 c.c. of 2 per cent by volume sulphuric acid for two hours. To the resulting solution 1 litre of 2 per cent sulphuric acid containing 15 gm. of silver sulphate was added while hot. After twenty-four hours the large precipitate of purine silver was filtered out and carefully washed. This silver precipitate was decomposed with hydrochloric acid and the solution made up to 1 litre. By determining the nitrogen in an aliquot part, this solution of the purines was found to contain 2.06 gm. of nitrogen. The filtrate from the silver precipitate was freed from silver with hydrogen sulphide, evaporated to 1 litre and 25 c.c. of it distilled with magnesium oxide. The ammonia evolved was 0.0014 gm., corresponding to 0.0560 gm. in the entire solution. The solution from which this ammonia was distilled was then subjected to a Kjeldahl nitrogen determination, and 1.1000 gm. nitrogen found to be present in the whole solution. The original solution of the nucleic acid therefore contained 3.2160 gm. of nitrogen. Ten sixteenths of this is 2.01 gm., which agrees closely with the 2.06 gm. found in the purine solution and

also with the previous determinations of the purines by direct weighing which have been made in this laboratory. The main solution containing the remainder of the nucleic acid was evaporated until it contained 20 per cent by volume of sulphuric acid and then heated for two hours in an autoclave at  $150^{\circ}$ . The solution was filtered from the large mass of humus that had formed which, when washed and dried, weighed 3.6875 gm. equal to 3.7797 gm. allowing for the solution used for the nitrogen determination, and contained 0.0802 gm. of nitrogen. The filtrate and washings of the humus were made up to 1 litre and found to contain practically the same quantity of ammonia as before heating at  $150^{\circ}$ , namely, 0.0533 gm. The slight difference is due to the unavoidable errors of analysis, which in this case were multiplied by 40. The remainder of the solution was then treated with phosphotungstic acid, the resulting precipitate filtered out, decomposed with baryta, and 0.8892 gm. of crude cytosine obtained. When once recrystallized, this substance separated in plates characteristic of cytosine which browned at about  $290^{\circ}$  and decomposed at  $320^{\circ}$ .

The air-dry substance when dried at  $110^{\circ}$  yielded 14.06 per cent of water, and the anhydrous substance 37.48 per cent of nitrogen.

*Water*, 0.2560 gm. subst. air dried, lost 0.0360 gm.  $H_2O$  at  $110^{\circ}$ .

Calculated for  $C_4H_5ON_2 \cdot H_2O = H_2O$  13.97 per cent.

Found . . . . . =  $H_2O$  14.06 " "

*Nitrogen*, 0.0960 gm. subst., dried at  $110^{\circ}$ , required 25.7 c.c.  $n/10$  HCl.

Calculated for  $C_4H_5ON_2 = N$  37.83 per cent.

Found . . . . . =  $N$  37.48 " "

The filtrate from the phosphotungstic acid precipitate was freed from phosphotungstic and sulphuric acids with baryta and from baryta with carbonic acid. The solution and washings from the barium carbonate were concentrated to 500 c.c. and nitrogen determined in 25 c.c. of the solution. The amount found corresponded to 0.4000 gm. of nitrogen in the whole solution, or, allowing for the solution used for the previous nitrogen determinations, to 0.4200 gm. This quantity is approximately two sixteenths of the nitrogen of the nucleic acid which was hydrolyzed, namely, 0.4020 gm. On concentrating this solution uracil separated in characteristic crystals which weighed 0.7325 gm., and from the mother liquor by precipitating with silver 0.1300 gm. more was obtained.

After once recrystallizing, this uracil decomposed at  $334^{\circ}$  and contained 24.70 per cent of nitrogen.

*Nitrogen*, 0.1100 gm. subst. required 19.4 c.c.  $n/10$  HCl.

Calculated for  $C_4H_4O_2N_2 = N$  25.00 per cent.

Found . . . . = N 24.70 " "

The total amount of uracil obtained, allowing for the solution used for the nitrogen determinations, was equivalent to 0.9508 gm. This quantity is probably less than that contained in the solution, as, owing to an accident, a large part of the final solution obtained from the silver precipitate was lost.

The following table shows the distribution of nitrogen at the different stages of the preceding operations:

After hydrolyzing at  $100^{\circ}$  with 2 per cent  $H_2SO_4$ ,

Purine nitrogen . . . .	2.0600 gm.
Ammonia nitrogen . . . .	0.0560 "
Other nitrogen . . . .	1.1000 "
Total nitrogen . . . .	3.2160 "

After hydrolyzing at  $150^{\circ}$  with 20 per cent  $H_2SO_4$ ,

Ammonia nitrogen . . . .	0.0533 gm.
Humus nitrogen . . . .	0.0802 "

After precipitating with phosphotungstic acid,

Nitrogen . . . . .	0.4200 gm.
Nitrogen precipitated by phosphotungstic acid	0.5998 "

From these figures it appears that, leaving out of consideration the small amount of ammonia, nearly three sixteenths of the nitrogen was precipitated with phosphotungstic acid and a little more than two sixteenths was not thus precipitated. This would indicate the presence of one molecule of cytosine and one of uracil for every four atoms of phosphorus in the nucleic acid. It is to be noted, however, that the actual quantity of cytosine obtained, 0.8892 gm., was much less than that thus calculated from the nitrogen precipitated with phosphotungstic acid, namely, 1.49 gm., and there may well be serious doubt as to whether such an amount was really present. Cytosine phosphotungstate, however, is difficult to decompose with baryta, and it was found that further digestion of this

precipitate with hot baryta solution did, in fact, yield a little more substance which, as it formed a picrate, makes it probable that we did not obtain all of the cytosine that was present. The amount of uracil, 0.9508 gm., also fell short of that calculated from the nitrogen in the solution, namely, 1.68 gm., but as there was a notable loss of the solution containing this substance, we certainly failed to secure all that was present.

From these results and those obtained earlier<sup>3</sup> it appears that fifteen sixteenths of the nitrogen of the nucleic acid probably belongs to guanine, adenine, cytosine, and uracil, of which one molecule of each is apparently present for every four atoms of phosphorus. The other one sixteenth of the nitrogen remains to be accounted for. Of this 0.0400 gm. was present in the silver precipitate in excess of the amount corresponding to ten sixteenths. This quantity may well be due to the difficulty of washing this bulky and gelatinous precipitate completely free from all other substances; 0.0560 gm. was ammonia nitrogen; 0.0802 gm. was in the humus, and 0.0180 gm. was in the uracil solution above the quantity calculated for two sixteenths, doubtless due to not quite complete precipitation of cytosine. The sum of these quantities is 0.1942 gm., from which should be deducted 0.0032 gm. for the amount by which the nitrogen of the phosphotungstic acid precipitate fell short of that calculated for three sixteenths, leaving a total of 0.1910 gm. accounted for against 0.2010 gm. calculated. The deficit of 0.0100 gm. is readily explained by the fact that the errors of several of the nitrogen determinations were multiplied by large factors.

This nitrogen, not belonging to the purines and pyrimidines, probably belongs to some other still unknown decomposition product, although it is not impossible that it may belong to a substance or substances which contaminated the preparation of nucleic acid. In view of the practical impossibility of making preparations of nucleic acid free from inorganic bases, it is easy to conceive that even the most carefully purified preparations of this acid may still contain nitrogenous bases as well.

<sup>3</sup> OSBORNE and HARRIS: Report of the Connecticut Agricultural Experiment Station for 1901, p. 365; also *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 85.

## THE INCREASE IN THE OSMOTIC CONCENTRATION OF THE BLOOD DURING ETHER AND CHLORO- FORM ANÆSTHESIA.

BY A. J. CARLSON AND A. B. LUCKHARDT.

[*From the Hull Physiological Laboratory, the University of Chicago.*]

IN a recent paper by Carlson, Greer, and Becht<sup>1</sup> it was shown that the osmotic pressure of the blood of dogs under ether narcosis and of horses under chloroform narcosis, as determined by the freezing-point method, was usually considerably greater at the end of an experiment than at the beginning. Since this increase in osmotic concentration of the blood was too great to fall within the limit of experimental error, a study of the cause of this variation was undertaken with the hope that the mechanism effecting this change in concentration might be discovered.

Though the differences in osmotic concentrations which they obtained were in most of the experiments very marked, their data are not conclusive. In many of their experiments, lasting at times more than three hours, there was elimination of saliva and cervical lymph, and since chorda saliva and especially the saliva following an injection of pilocarpin is not only abundant but also very dilute, it is possible that the increased concentration of the blood was simply consequent to the large elimination of water by the salivary glands.

### I. EXPERIMENTAL METHODS.

In the first three experiments (Table I, 1, 2, 3,) the animals were lightly anæsthetized with ether before drawing the first sample of blood. A cannula was inserted into the femoral artery and 10-15 c.c. of blood were collected into graduates and defibrinated by shaking with a few pieces of glass. During the course of the anæsthesia, usually lasting from two to two and a half hours, the ether vapor was administered in the ordinary way through a tracheal cannula.

<sup>1</sup> A. J. CARLSON, J. R. GREER, and F. C. BECHT: *This journal*, xix, 1907, pp. 377, 379.

At the end of the experiment another sample of 15 c.c. of blood was drawn and defibrinated in the same manner. In order to obtain the blood before the animal was subjected to the anaesthetic the procedure in Experiments 4 to 13 was slightly different. The skin and subcutaneous tissue over the course of the femoral artery was anaesthetized by means of ethyl chloride before exposing the artery and drawing the blood. In Experiments 9 and 10 a subcutaneous injection of cocaine along the course of the femoral artery was given in place of the ethyl chloride.

The animals used were large cats and medium-sized dogs. Ether was used in the first ten experiments; in Experiments 10 and 12 chloroform was employed, and in Experiment 13, morphine chloroform. The ordinary Beckmann apparatus was employed for the freezing-point determination.

## II. RESULTS.

It will be seen from Table I that in ten out of the thirteen experiments the blood showed an increased osmotic concentration at the end of the anaesthesia. The increase of  $\Delta$  varied between  $0.004^\circ$  and  $0.086^\circ$ , the average increase in the depression of the freezing-point being about  $0.036^\circ$ . The only strikingly negative result was obtained in Experiment 3, where the decrease of  $\Delta$  at the end of the ether anaesthesia amounted to  $0.053$ .

On the whole, it may therefore be safely said that ether and chloroform anaesthesia increase the osmotic concentration of the blood serum.

There are a number of possible causes which might contribute to this increase in concentration:

1. First of all, there might be a lack of compensation by the tissues for the water lost in the expired air. It is conceivable that the partially anaesthetized tissues, the kidneys included, respond less readily to the osmotic change of the blood due to evaporation from the lungs and skin. If this is a factor, we would expect the osmotic concentration to be directly proportional to the duration of the anaesthesia, but this does not appear to be the case in our experiments.

2. There might be a complete or partial suppression of the urine during the course of the anaesthesia. This would increase the osmotic concentration of the serum.<sup>2</sup> In some of the dogs this was

<sup>2</sup> SEGALÉ: *Archives italiennes de biologie*, 1907, xlvii, p. 373.



the case, though this was never true in the experiments on cats. On the other hand, this suppression of urine formation might be counterbalanced by a diminished metabolism of the body in general. Hence the mere fact of partial suppression of the urine does not prove that this is the cause of the increased osmotic concentration.

TABLE I.

The increase in osmotic concentration of the serum in ether and chloroform narcosis. In Experiments 1, 2, and 3 the animals were under light narcosis at the time of drawing the first sample of blood. In the remaining experiments the first blood sample was drawn before administration of the anæsthetic.

Exp.	Animal.	$\Delta$ of blood before narcosis.	Length of narcosis.	$\Delta$ of blood after narcosis.	Increase of $\Delta$ .	Decrease of $\Delta$ .	Narcotic.
I.	Dog	0.620	120 min.	0.630	0.010	....	Ether
II.	Cat	0.678	150 min.	0.728	0.050	....	Ether
III.	Cat	0.720	150 min.	0.667	....	0.053	Ether
IV.	Cat	0.655	120 min.	0.681	0.026	....	Ether
V.	Cat	0.693	150 min.	0.720	0.027	....	Ether
VI.	Cat	0.659	150 min.	0.689	0.030	....	Ether
VII.	Cat	0.633	120 min.	0.719	0.086	....	Ether
VIII.	Cat	0.693	120 min.	0.690	....	0.003	Ether
IX.	Dog	0.639	15 min.	0.690	0.051	....	Ether
X.	Dog	0.603	120 min.	0.651	0.048	....	Ether
XI.	Cat	0.660	30 min.	0.666	0.006	....	Chloroform
			120 min.	0.664	0.004	....	
XII.	Cat	0.702	45 min.	0.666	....	0.036	Chloroform
			90 min.	0.684	....	0.018	
XIII.	Dog	0.613	105 min.	0.642	0.029	....	Morphine-chloroform
			150 min.	0.639	0.026	....	

3. That hyperglycemia may be a factor in causing the increased concentration of the blood during anæsthesia in cats is possible, since the cat's urine at the end of the anæsthesia invariably shows an abundance of sugar when tested with Fehling's solution. Normal cat's urine gives no sugar test with Fehling's solution. But ordinary ether anæsthesia does not produce hyperglycemia in dogs, although it effects osmotic concentration.

4. Owing to the hemolytic action of ether and chloroform, salts

may be liberated from the corpuscles of the blood. Deep anæsthesia bordering on death is accompanied by some laking both in dog and cat. But ether narcosis sufficiently deep to abolish the corneal reflex usually does not cause any laking, at least during the first hour. Unless the anæsthetics act on the corpuscles in a way to liberate the salts before the liberation of the hemoglobin, the osmotic concentration of the serum in anæsthesia cannot be the result of hemolysis, although it may be one of the factors in deep anæsthesia.

5. Alcock<sup>3</sup> found that chloroform acting on the skin of the frog caused a diminution of electrical resistance of about 24 per cent and of polarization of about 10 per cent. This observer believes that the action of chloroform, namely, "the breaking down of a semi-permeable apparatus, is the characteristic action of an anæsthetic on living tissues." If this is the case, water would pass from the blood into the tissues, and some of the salts would probably pass from the tissues into the blood, since the osmotic concentration of tissue cells is higher than the osmotic concentration of the blood serum.

Diffusion of the electrolytes from the tissue cells into the lymph and blood stream during anæsthesia may be effected by liberation of electrolytes within the cells without any change in the permeability of the cell walls. Roaf and Alderson<sup>4</sup> have recently published results which show that chloroform, ether, carbon dioxide, acetic acid, and heat coagulation cause a physical change in the condition of the inorganic constituents of tissues. This change consists, at least in part, in a liberation of electrolytes. Under these conditions salts are detached from erythrocytes, minced brain, liver, muscle, and kidney, *but not from the constituents of blood serum*. That blood serum sets free no salts when thus treated remains a significant fact. If only moderate quantities of the anæsthetics had been used by Roaf and Alderson, their results might have been applicable to conditions of normal anæsthesia. To 50 c.c. or 50 gm. of the minced tissue they added as much as 20–30 c.c. of ether. When such quantities of ether or chloroform are used, we cannot be certain that we are dealing with maximum effects of ordinary anæsthesia. According to Buckmaster and Gardner<sup>5</sup> the amount

<sup>3</sup> ALCOCK: Proceedings of the Royal Society, 1906, lxxviii, p. 159.

<sup>4</sup> ROAF and ALDERSON: Biochemical journal, 1907, ii, p. 412.

<sup>5</sup> BUCKMASTER and GARDNER: Proceedings of the Royal Society, 1906, lxxvii, B, p. 414.

of chloroform in the blood at the disappearance of the conjunctival reflexes in cats amounts to 14-27 mg. per 100 gm. of blood, and only rises to 40 mg. per 100 gm. of blood at the cessation of respiration. Nicloux<sup>6</sup> found that the amount of ether per 100 gm. of liver, brain, kidney, and muscle never rises above 176 mg. at the moment of the death of the animal, and that the amount of the anæsthesia in the blood at the time of death never rises above 170 mg. per 100 c.c.<sup>7</sup> The large quantities of ether and chloroform used by Roaf and Alderson evidently killed and partly dissolved the tissues, thus liberating some of the salts contained in the cell. This view is further strengthened by the fact that no electrolytes were split off when the ether and chloroform were added to blood serum.

6. Finally, there remains the possibility that the increased osmotic concentration of the blood after narcosis is due to the osmotic pressure of the anæsthetic dissolved in the serum. Three experiments (Table I, Exp. 11, 12, and 13) showed that the length of the narcosis has no appreciable effect on the concentration, inasmuch as samples of blood drawn at the end of two hours were less concentrated than those drawn during the first half or one and three-fourths hours of anæsthesia. These facts, together with the result of Experiment 9, where an increase of 0.051 of  $\Delta$  followed a fatal anæsthesia of fifteen minutes, suggests the possibility that the increased concentration of the blood varies directly with the depth of the anæsthesia, and might be due entirely to the osmotic pressure of the anæsthetic itself.

To determine what effect pure ether has in lowering the freezing-point of distilled water, 1, 2, 3, and 4 drops were added to as many flasks containing 25 c.c. of water. Even at this dilution the ether causes a lowering of the freezing-point averaging about 0.016° per drop (Table II). Three series of experiments were carried on with ether at a still greater dilution. One, 2, 3, 4, and sometimes 5 drops of ether were added to as many flasks, each containing 50 c.c. of distilled water. As can be gathered from an inspection of Table II, the average depression of  $\Delta$  per drop of ether at this dilution amounted to 0.008°.

The increased osmotic concentration of the blood of an animal under ether and chloroform anæsthesia may thus be due merely to the anæsthetic dissolved in the serum. If this is the main factor,

<sup>6</sup> NICLOUX: *Comptes rendus*, 1907, cxliv, p. 341.

<sup>7</sup> NICLOUX: *Comptes rendus de la Société de biologie*, 1906, lxi, pp. 228-231.

the osmotic concentration of the blood, as determined by the freezing-point determination, ought to vary directly with the depth of anæsthesia at the time blood is drawn.

To test this hypothesis on the living animal 15 c.c. of blood was drawn without anæsthesia from the femoral artery of a large dog, in the manner described above. The  $\Delta$  of this blood was  $0.648^{\circ}$ . The dog was now anæsthetized with ether so deeply that he had to be resuscitated by means of artificial respiration after the second sample of 15 c.c. of blood had been drawn. The  $\Delta$  of this sample

TABLE II.

THE LOWERING OF THE FREEZING-POINT OF DISTILLED WATER BY ADDITION OF VARYING QUANTITIES OF ETHER.

Ether.	25 c.c. H <sub>2</sub> O. Experiment I.		50 c.c. H <sub>2</sub> O.					
			Experiment II.		Experiment III.		Experiment IV.	
....	$\Delta$	$\Delta$ per drop.	$\Delta$	$\Delta$ per drop.	$\Delta$	$\Delta$ per drop.	$\Delta$	$\Delta$ per drop.
1 drop	0.018	0.018	0.006	0.006	....	....	....	....
1 drop	0.016	0.016	0.007	0.007	0.010	0.010	0.008	0.008
2 drops	0.027	0.014	0.017	0.008	0.017	0.008½	0.017	0.008½
3 drops	0.053	0.018	0.027	0.009	0.027	0.009	0.022	0.007½
4 drops	0.065	0.016	....	....	....	....	0.028	0.007
5 drops	....	....	....	....	....	....	0.036	0.007
Average $\Delta$ per drop: 0.016½			Average $\Delta$ per drop: 0.008					

was  $0.713^{\circ}$ , — an increase of  $0.065^{\circ}$  in the fifteen minutes of deep anæsthesia. The dog was now kept under light ether anæsthesia for one hour. The ether was completely cut off, and when the animal became sufficiently conscious to readily follow the movement of the hand as it passed from side to side before its eyes, a third sample of blood was drawn from the femoral artery. The  $\Delta$  of the blood had fallen to  $0.653^{\circ}$  from  $0.713^{\circ}$ . The dog was again put under deeply by vigorously pushing the anæsthetic. In fifteen minutes, when sample 4 was taken, respiration was very

feeble. The  $\Delta$  of this sample was  $0.722^\circ$ . The anæsthetic was pushed still more. After drawing off the last sample the respiration had stopped and the animal had to be resuscitated. The blood was venous and gave as  $\Delta$ ,  $0.737^\circ$ . These variations are represented graphically in Fig. 1, the  $\Delta$  of the various samples being plotted along the vertical, the time of drawing the various samples, 1, 2, 3, 4, and 5, being plotted along the horizontal line.

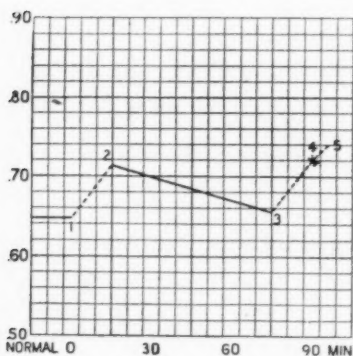


FIGURE 1.—Curve of variation in the osmotic concentration of the serum with the variation in the degree of anæsthesia. The periods of deep anæsthesia are indicated by the broken line, the period of light anæsthesia by the continuous line. The figures on the curve indicate the time of drawing of the blood samples.

The fact that the osmotic concentration varies with the depth of the anæsthesia is, however, not in itself a conclusive proof that this concentration is solely due to the anæsthetic dissolved in the serum, as the other possible factors—laking, change in permeability of the tissue cells, etc.—would also vary directly with the degree of anæsthesia. Burton-Opitz<sup>8</sup> has shown that deep ether and chloroform narcosis increases the viscosity of the blood, indicating an increase in the percentage of the organic constituents in the blood, or some change in the physical state of these constituents.

### III. SUMMARY AND CONCLUSIONS.

1. During chloroform and ether anæsthesia there is an increase in the osmotic concentration of the blood as compared to that of the normal blood. This increase in the osmotic concentration appears to be directly proportional to the depth of the anæsthesia and independent, at least to a considerable extent, of the duration of the anæsthesia.

2. The main factor in this increased osmotic concentration is the ether or chloroform itself dissolved in the serum. Other factors, discussed in the body of this report, can, however, not be excluded.

<sup>8</sup> BURTON-OPITZ: *Journal of physiology*, 1905, xxxiii, p. 385.

## A NOTE ON THE UNION OF THE PROTEINS OF SERUM WITH ALKALI.

By LAWRENCE J. HENDERSON.

[*From the Laboratory of Biological Chemistry in the Harvard Medical School.*]

ALTHOUGH it is now more than half a century since the possibility of union of proteins with acids was first suggested,<sup>1</sup> and though their capacity to combine with acids and alkalis has long been established and quantitatively studied,<sup>2</sup> we are still ignorant of the degree to which, in the body, alkali compounds of proteins may exist, as a result of the very faint preponderance of alkalinity in blood and probably also in protoplasm.

The matter of the compounds of the plasma proteins with alkali is of special importance in the theory of carbonic acid transport, for it is now generally believed, in accordance with the experiments of Sertoli and Hoppe-Seyler and the clear discussion of Zuntz,<sup>3</sup> that the passing off of carbonic acid from the blood involves a transfer of sodium from carbonic acid to protein. Indeed we are at a loss how else to explain this highly important phenomenon, in that no other substance is known to occur in blood plasma in sufficient amount to play an important rôle in this matter.<sup>4</sup>

In view of the extreme delicacy of adjustment of such equilibria in the organism, it may well seem a hopeless task accurately to characterize this equilibrium, where one of the factors in the reaction is a mixture of indeterminate composition and doubtful physical state. None the less it is clear that present methods hold out hope of at least an approximate determination of the rôle of the proteins in this important matter, and it may be said with confidence that our scanty knowledge of protein alkali compounds in their quantitative rela-

<sup>1</sup> C. SCHMIDT: *Annalen der Chemie und Pharmacie*, 1847, lxi, p. 317.

<sup>2</sup> By RICHET, F. A. HOFFMANN, SJOQVIST, BUGARSKY and LIEBERMANN, HARDY, and others.

<sup>3</sup> See the article by ZUNTZ in HERMANN's *Handbuch*, vol. iv, ii, p. 64.

<sup>4</sup> See the article by BOHR in NAGEL'S *Handbuch*, i, pp. 180 ff.

tionships is not inconsistent with the ideas which are forced upon us *a priori* by our knowledge of carbonic acid transport.

This paper presents a report of certain simple preliminary experiments which, on the probably safe assumption that the serum proteins behave with alkalis, on the whole, like simple substances in true solution, indicate the magnitude of the union between such substances in very faintly alkaline solutions. The results offer nothing more than a first approximation to a characterization of the complicated equilibrium involved, and they are presented only because such knowledge is wholly lacking.

#### EXPERIMENTAL PART.

Three samples of fresh blood serum from the pig were mixed with large quantities of toluol and permitted to dialyze during periods of from eight to ten days against running water. Once in twenty-four hours 100 c.c. of saturated neutral sodium chloride solution per litre of serum were added to the fluids. At the end of the dialysis the liquids were perfectly clear, and in only one case was there a measurable precipitate of globulin. In all three cases it was found necessary to add small but appreciable quantities of alkali to the solutions in order to obtain the neutral reaction with rosolic acid. This indicates conclusively that the diffusible alkaline substances of the serum had been effectively removed by the process of dialysis, and it presents a sufficient guarantee that not more than traces of protein alkali compounds can have been present.

In such preparations as these it may be assumed that after the addition of sufficient alkali to make the reaction precisely neutral, the only substances present which can still combine with alkalis in appreciable amount are the serum proteins. Accordingly the power of these solutions to neutralize sodium hydrate may be regarded as a measure of the sodium protein compounds of the blood plasma, fibrinogen being disregarded.

The determination of the combining power of the preparations with sodium hydrate were carried out according to the method of Salm, which is probably, in cases such as this, nearly if not quite as accurate as the ordinary determinations based upon measurements of electromotive force. The procedure consisted in diluting 10 c.c. portions of the solutions to 500 c.c. with dilute sodium chloride solution,<sup>5</sup> and titrating to the neutral point as indicated by rosolic acid

<sup>5</sup> It was found that dilution did not appreciably affect the end point of the titration.



and to the first faint coloration with phenolphthalein, which indicates a hydrogen ionization of approximately  $0.03 \times 10^{-7}$ . The difference between these two titrations probably indicates pretty accurately the amount of sodium in combination with protein at the above-mentioned hydrogen ionization, that is to say, when the hydroxyl ionization is about  $20 \times 10^{-7}$ .

The observations are as follows, the sodium hydrate solution being 0.061 N.

PREPARATION I.

Concentration of proteins 3.5 per cent. — Alkali required to obtain neutral reaction with rosolic acid:

1.10 c.c.	1.08 c.c.
1.12 c.c.	Av. 1.10 c.c.

Alkali required to obtain coloration of phenolphthalein:

1.70 c.c.	1.73 c.c.
1.70 c.c.	Av. 1.71 c.c.

PREPARATION II.

Concentration of proteins 1.2 per cent. — Alkali required to obtain neutral reaction with rosolic acid:

0.35 c.c.	0.35 c.c.
0.40 c.c.	Av. 0.37 c.c.

Alkali required to obtain coloration of phenolphthalein:

0.60 c.c.	0.60 c.c.
0.55 c.c.	0.55 c.c.
Av. 0.58 c.c.	

PREPARATION III.

Concentration of proteins 2.0 per cent. — Alkali required to obtain neutral reaction with rosolic acid:

0.40 c.c.	0.40 c.c.
0.40 c.c.	Av. 0.40 c.c.

Alkali required to obtain coloration of phenolphthalein:

0.75 c.c.	0.75 c.c.
0.80 c.c.	Av. 0.77 c.c.

These results indicate that in an 8 per cent solution of the proteins, such as exists in blood, the amount of alkali in combination with proteins at a hydroxyl ionization of about  $20 \times 10^{-7}$  is:

I. . . . .	0.0085 N
II. . . . .	0.0085 N
III. . . . .	0.0090 N
Average . . . . .	0.0088 N

The hydroxyl ionization in blood is about one tenth of that for which the above results are calculated, and for small changes in hydroxyl ionization the concentration of alkali protein compounds probably varies not far from proportionately to the variation in the hydroxyl ionization. The outcome of this investigation, then, is that the concentration of sodium protein compounds in normal blood plasma at  $18^{\circ}$  is not far from 0.001 N.

This concentration, however, is probably only about 1/20 of the concentration of sodium bicarbonate in blood, and it does not seem probable that the diminution in sodium bicarbonate content of blood should be accompanied by a four or five fold increase in the sodium protein compounds when carbonic acid is given off in the lung; for that would involve a four or five fold increase in the hydroxyl ionization.

Evidence has been obtained in this laboratory, however, that there is a material increase in the alkalinity of blood as the temperature rises, and that the alkalinity of blood at body temperature is several times greater than at  $18^{\circ}$ . If this change in hydroxyl ionization with the temperature is accompanied by a corresponding increase in the sodium protein compounds, the concentration of the latter substances in blood in the body would become so much greater than above calculated as to correspond to expectations based upon the theory of carbonic acid transport. In any case it is clear from this investigation that the concentration of sodium protein compounds in blood is at least of the order of magnitude which was to be expected, and that such compounds can play an important rôle in carbonic acid excretion.

# CONCERNING THE RELATIONSHIP BETWEEN THE STRENGTH OF ACIDS AND THEIR CAPACITY TO PRESERVE NEUTRALITY.

BY LAWRENCE J. HENDERSON.

[From the Laboratory of Biological Chemistry in the Harvard Medical School.]

IN the light of relationships already discussed concerning the mechanism for the preservation of neutrality in the animal organism,<sup>1</sup> it is interesting to consider what the principles underlying the regulation of the reaction of a simple solution may be, for in this way it may be possible to discover how closely the physiological mechanism approaches to ideal conditions.

According to the mass law, in the pure solution of a weak acid, HA, the relationship holds, —

$$k \cdot (HA) = (\overset{+}{H}) \cdot (\overset{-}{A}),$$

where  $k$  is the ionization constant of the acid and the enclosed quantities represent the concentrations of the respective substances. This equation may be more conveniently written

$$(\overset{+}{H}) = k \cdot \frac{(HA)}{(\overset{-}{A})}.$$

If now the sodium salt, or other salt with a strong base, of the acid be introduced into such a solution as is above considered, it is evident that we shall have the following condition. The concentration of unionized molecules of acid will be almost precisely equal to the total amount of acid present, while the concentration of the ions ( $\overset{-}{A}$ ), much increased because of the presence of the salt, will be equal to the concentration of the salt multiplied by its degree of dissociation. This latter factor, varying with the strength of the acid and the concentration of the salt, will usually not be less than 0.6.

We may therefore write the equation, —

<sup>1</sup> See HENDERSON and BLACK: This journal, 1907, xviii, pp. 250-255.

$$(\bar{H}) = K \cdot \frac{HA}{MA},$$

where  $K$  is the ionization constant of the acid divided by the degree of ionization of the salt, and  $HA$  and  $MA$  represent the amounts of acid and salt present in the solution.

From an inspection of the above equation it is easy to see that if  $K$  is equal to the hydrogen ionization at neutrality, approximately  $1 \times 10^{-7}$ , the conditions will be exceedingly favorable for the maintenance of neutrality. For at neutrality the ratio between acid and salt must then be equal to 1, and accordingly the addition of acid or alkali will here influence but slightly the magnitude of the ratio, at least at first, thereby influencing but slightly the hydrogen ionization.<sup>2</sup>

It may readily be shown that in general the preservation of neutrality within any range of acidity and alkalinity is most efficient when  $K$  is equal to the square root of the water constant.

Let  $K = k \cdot \sqrt{C_{H_2O}}$ , when  $C_{H_2O}$  is the water constant.

Then  $(\bar{H}) = \frac{HA}{MA} \cdot k \cdot \sqrt{C_{H_2O}}$ ,

but  $(\bar{H}) = \frac{C_{H_2O}}{(OH)}$ ;

hence  $(OH) = \frac{MA}{HA} \cdot \frac{1}{k} \cdot \sqrt{C_{H_2O}}$ .

Let  $\frac{HA}{MA} = R$  (the ratio between acid and salt).

Then (I)  $(\bar{H}) = R \cdot k \cdot \sqrt{C_{H_2O}}$ .

(II)  $(OH) = \frac{1}{R} \cdot \frac{1}{k} \cdot \sqrt{C_{H_2O}}$ .

The rate of change in hydrogen ionization as  $R$  changes may be found by differentiating equation (I) with respect to  $R$ , and

<sup>2</sup> This conclusion, developed in wholly different connections, was presented at a meeting of the Physico-Chemical Club of Boston and Cambridge simultaneously by E. W. WASHBURN and myself; see E. W. WASHBURN, *Journal of the American Chemical Society*, 1908, xxx, p. 31.

similarly the rate of change in hydroxyl ionization as  $\frac{1}{R}$  changes by differentiating equation (II) with respect to  $\frac{1}{R}$ . Thus we obtain the equations, —

$$(III) \quad \frac{d(\bar{H})}{dR} = k \cdot \sqrt{C_{H_2O}}.$$

$$(IV) \quad \frac{d(\bar{OH})}{d\frac{1}{R}} = \frac{1}{k} \cdot \sqrt{C_{H_2O}}.$$

Clearly, for the most efficient preservation of neutrality, the sum of these two rates of change must be as small as possible. Representing the sum of the rates of change by  $S$ , we may write the equation, —

$$(V) \quad S = k \cdot \sqrt{C_{H_2O}} + \frac{1}{k} \cdot \sqrt{C_{H_2O}}.$$

The condition that  $S$  may be as small as possible may be found by differentiating with respect to  $K$  and putting the derivative equal to 0; that is to say,

$$\left(1 - \frac{1}{k^2}\right) \cdot \sqrt{C_{H_2O}} = 0,$$

$$\text{whence} \quad (VI) \quad k = 1.$$

$$(VII) \quad \begin{cases} K = \sqrt{C_{H_2O}} \\ K_{24^\circ} = 1 \times 10^{-7}. \end{cases}$$

This theoretical conclusion may be readily verified with a moderate but sufficient degree of accuracy by simple titration experiments with properly chosen indicators, in accordance with the recent studies of Salm.<sup>3</sup> Thus, using solutions of the acids mono-sodium phosphate, carbonic acid, acetic acid, hydrogen sulphide, picolinic acid, boric acid, and phenol, I have found that to pass from one standard coloration with rosolic acid to another standard coloration, it is necessary to use the greatest amount of alkali for hydrogen sulphide; mono-sodium phosphate and carbonic acid follow closely, and the other substances at a greater distance.

The experiments were carried out by simple titration, using solutions that varied between 0.04 N and 0.12 N<sup>4</sup> in individual cases.

<sup>3</sup> E. SALM: Zeitschrift für physikalische Chemie, 1906, lvii, p. 471.

<sup>4</sup> The acid substances are all regarded as mono-basic.

The colors were always compared with standard colors given by mixtures of mono- and di-sodium phosphate, and at least three determinations were made in every case. No difficulty was experienced save in the case of hydrogen sulphide, where it was finally found better to titrate from the color with neutral red given by a solution of mono- and di-sodium phosphates in the ratio 1 : 1 to the color given by a similar solution in the ratio 1 : 6.7 with rosolic acid. The data of these determinations are presented as an example of the perfectly simple procedure:

NaOH . . . . .	0.102 N.		
NaH <sub>2</sub> PO <sub>4</sub> . . . . .	0.082 N.		
H <sub>2</sub> S . . . . .	0.045 N.		
	First color.		Second color.
10 c.c. NaH <sub>2</sub> PO <sub>4</sub> . . . . .	4.00 c.c. NaOH		7.00 c.c. NaOH.
10 c.c. H <sub>2</sub> S . . . . .	1.55 c.c. NaOH		3.30 c.c. NaOH.
10 c.c. H <sub>2</sub> S . . . . .	1.15 c.c. NaOH		3.35 c.c. NaOH.
10 c.c. H <sub>2</sub> S . . . . .	1.15 c.c. NaOH		3.00 c.c. NaOH.
10 c.c. H <sub>2</sub> S . . . . .	1.35 c.c. NaOH		3.00 c.c. NaOH.

On the average, as appears from the above table, the first color was given by a solution containing hydrogen sulphide and its acid sodium salt in the ratio 1 : 0.42, and the second color by a similar mixture in the ratio 1 : 2.5.

In the following table are presented the results of these titrations, expressed in the form of the relative amounts of alkali required for equivalent solutions of the different acids to pass from the one standard coloration to the other, the amount of alkali required for mono-sodium phosphate being used as the basis for comparison.

Acid.	$K \times 10^7$ .	Alkali required.
Phenol . . . . .	0.0013	0.01
Boric acid . . . . .	0.017	0.08
Hydrogen sulphide . . . . .	0.57	1.10
Mono-sodium phosphate . . . . .	2.0	1.00
Carbonic acid . . . . .	3.0	0.72
Picolinic acid . . . . .	18.0	0.10
Acetic acid . . . . .	180.0	0.03

These relationships may readily be compared with the theory presented above by a slightly different analysis of the relationships. The equation

$$(\overset{+}{H}) = \frac{HA}{MA} \cdot k \cdot \sqrt{C_{H_2O}}$$

may conveniently be written

$$(\overset{+}{H}) = \frac{HA}{MA} \cdot k \cdot 10^{-7}.$$

Let

$$HA + MA = 1$$

$$(\overset{+}{H}) = \frac{1 - MA}{HA} \cdot k \cdot 10^{-7}.$$

Let it be required to find the amount of alkali,  $X$ , required to convert a mixture of acid and salt of hydrogen ionization  $2 \times 10^{-7}$  into a mixture of hydrogen ionization  $0.5 \times 10^{-7}$ .

$$\frac{1 - (MA)_1}{(MA)_1} \cdot k \cdot 10^{-7} = 2 \cdot 10^{-7}.$$

$$\frac{1 - (MA)_2}{(MA)_2} \cdot k \cdot 10^{-7} = \frac{1}{2} \cdot 10^{-7}.$$

$$2(MA)_1 + k(MA)_1 = k.$$

$$\frac{1}{2}(MA)_2 + k(MA)_2 = k.$$

$$(MA)_1 = \frac{k}{k + 2}.$$

$$(MA)_2 = \frac{k}{k + \frac{1}{2}}.$$

$$x = (MA)_2 - (MA)_1 = \frac{k}{k + \frac{1}{2}} - \frac{k}{k + 2}.$$

$$x = \frac{3}{5 + 2 \left( k + \frac{1}{k} \right)}$$

With the aid of this equation the following table is constructed:

$k$	$x$	$k$	$x$	$k$	$x$
$1 \cdot 10^{-8}$	0.0002	$4 \cdot 10^{-7}$	0.179	$5 \cdot 10^{-8}$	0.325
$1 \cdot 10^{-4}$	0.002	$3 \cdot 10^{-7}$	0.214	$4 \cdot 10^{-8}$	0.312
$1 \cdot 10^{-5}$	0.021	$2 \cdot 10^{-7}$	0.263	$3 \cdot 10^{-8}$	0.285
$1 \cdot 10^{-6}$	0.089	$1 \cdot 10^{-7}$	0.324	$2 \cdot 10^{-8}$	0.239
$9 \cdot 10^{-7}$	0.097	$9 \cdot 10^{-8}$	0.329	$1 \cdot 10^{-8}$	0.156
$8 \cdot 10^{-7}$	0.107	$8 \cdot 10^{-8}$	0.332	$1 \cdot 10^{-9}$	0.025
$7 \cdot 10^{-7}$	0.119	$7 \cdot 10^{-8}$	0.333	$1 \cdot 10^{-10}$	0.003
$6 \cdot 10^{-7}$	0.134	$6 \cdot 10^{-8}$	0.332	$1 \cdot 10^{-11}$	0.0003
$5 \cdot 10^{-7}$	0.153				

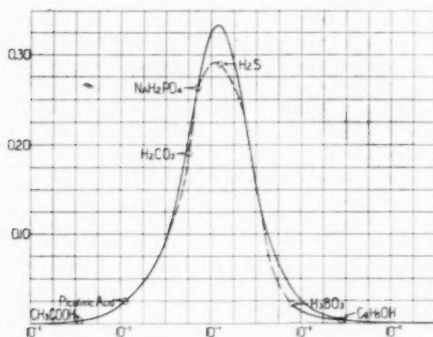
$k$  = ionization constant of acid.

$x$  = neutralizing power.



This table may be graphically presented. In the accompanying diagram the continuous line represents the calculated relationships of the table, and the dotted line the experimentally determined relationships.<sup>5</sup> The values of  $x$  in the table are plotted as ordinates, and values of  $k$  are plotted logarithmically as abscissas. It is evident that the accord between theory and observation is very good.

This quantitative analysis, theoretical and experimental, of the efficiency of neutrality preservation amply justifies the belief that



in the organism carbonic acid and phosphoric acid can serve very efficiently to preserve neutrality, and it shows that they possess high qualifications for the carrying out of this function. Needless to say, the conditions within the organism are more complicated than those in simple solution, but such simple

solutions as these which we are here considering furnish evidence which is directly applicable to the true solutions of the organism. The complications involved in these equilibria by the complexity of the organization of the animal body do indeed serve to modify conditions, in part by furnishing reservoirs of supply and vehicles of escape for the components of the simple systems. But, as I hope later to show, in great part these modifications of the conditions of equilibrium in the body serve greatly to increase the efficiency of carbonates and phosphates in preserving neutrality.

#### SUMMARY.

It appears that acids whose ionization constant is nearly equal to the hydrogen ionization at neutrality possess, with the help of their salts, a great capacity for preserving neutrality in simple solution, while other acids are in like concentration of relatively very little

<sup>5</sup> For purposes of comparison these are brought to the scale of the calculated relationships by assuming for mono-sodium phosphate the correct value. This assumption is amply justified by experiments now being carried on in this laboratory. This paper is, however, concerned only with relative magnitudes.

effect in this matter. Other things being equal, the greatest possible efficiency in preserving neutrality, on both sides of the neutral point, is possessed by that acid whose ionization constant is precisely equal to the hydrogen ionization of water divided by the degree of ionization of the salt. Clearly, then, the ionization constant of carbonic acid,  $3 \times 10^{-7}$ , and of the ion  $\text{H}_2\text{PO}_4$ ,  $2 \times 10^{-7}$ , gives them nearly the greatest possible efficiency for preserving neutrality in simple solution.

## THE REACTION OF BLINDED LOBSTERS TO LIGHT.

By PHILIP B. HADLEY.

[*From the Biological Laboratory of Brown University and the Wickford Experiment Station of the Rhode Island Commission of Inland Fisheries.*]

### I. INTRODUCTION.

IN an earlier paper<sup>1</sup> the writer has considered at length some points in the behavior of the American lobster in the larval and early adolescent stages.<sup>2</sup> The investigations here reported were incidental to a more extensive study of the reactions to light of normal lobsters. They are not assumed to be complete or conclusive, and it is merely the aim of the present paper to present briefly some characteristics of the behavior of the larval and early adolescent lobsters when one or both eyes have been blinded. The experiments were conducted at the Wickford Experiment Station of the Rhode Island Fish Commission, where, through the kindness of the director, Dr. A. D. Mead, facilities for work were placed at the disposal of the writer, and where it was made possible to secure large numbers of young lobsters in all of the early and some of the later stages.

The investigations of the writer upon the behavior of the larvæ and early adolescent lobsters have led him to believe that their reactions to light are determined by two conditions of illumination:

<sup>1</sup> HADLEY: Report of the Rhode Island Commission of Inland Fisheries for 1906.

<sup>2</sup> Briefly stated, these results, so far as they deal with reaction to light, showed that lobster larvæ in the first three stages reacted to light by placing the longitudinal axis of the body parallel to the direction of the light rays, with the head directed away from their source; and then by swimming, by means of the exopodites of the thoracic appendages, either toward or from that source. Any change in the direction of the light rays, striking the eyes of the larvæ, determined a new direction of swimming; and any change in the intensity of light might determine a change in the index of the reaction. Thus an intensity of light, which brought about a negative reaction, might, if increased, produce a positive response.

first, the purely directive influence, so called, which, by conditioning different degrees of illumination on each eye, may cause a definite body orientation; secondly, a tendency to select, in varying intensities of light striking both eyes equally, areas of optimal illumination, and to remain therein. Experiments to date do not lead the writer to believe that the reactions of the larvæ to the intensity of light (photopathy) are produced by means of many slight responses to the directive influence of the light, as was assumed by Yerkes<sup>3</sup> to be the case for *Daphnia pulex*. In consequence of this view the following experiments will be found to deal with three features of behavior: (1) the phototactic reaction, (2) the photopathic reaction, and (3) the method of reaction or the mechanics of orientation; and each of these points will be discussed in relation to three groups of larvæ: (1) Normal larvæ, (2) those with one eye blinded, and (3) those with both eyes blinded.

**Technique.** — The experiments, which were conducted with lobsters of the first five stages, were performed chiefly by daylight, in a room receiving light from the north and south, but so screened by dark curtains that the direction of the light rays could be accurately regulated. The larvæ, one or both of whose eyes had been excised by small scissors or blinded by a hot needle, were placed, as the experiments required, in one of two receptacles. — one a rectangular glass compartment  $6 \times 3 \times 2$  inches; the other a wooden box  $12 \times 6 \times 3$  inches, fitted with a glass bottom and a light-tight cover and painted black on the inside. During the experiments this box was set over a so-called light-shaft, which was built up from the laboratory table in such a way that parallel rays of light from a mirror, set at the base of the shaft, could be reflected up to the glass bottom of the box, which was placed 18 inches above the table, over the top of the shaft. By this contrivance all except nearly parallel rays of light were excluded, and these alone could influence the behavior of the larvæ swimming in the salt water, which was seldom more than 12 mm. deep.

The quality of light entering the bottom of the box was further modified by plates of red, orange, green, blue, or white glass, which were laid in linear series over the top of the light-shaft, and at the same time directly under the glass bottom of the box. These plates

<sup>3</sup> Reactions of *Daphnia pulex* to light and heat. Mark Anniversary volume, 1903, pp. 359-377.

of colored glass served to determine the intensity of light which was brought to bear on the larval lobsters, as they swam through different regions of the box. In other instances the light at opposite ends of the glass-bottomed box was regulated by graded wedge-shaped screens of gelatine which had been darkened with india ink. The resulting reactions of the larvæ were so similar and constant under both these conditions of illumination that any tendency on the part of the larvæ to gather at any particular part of the box

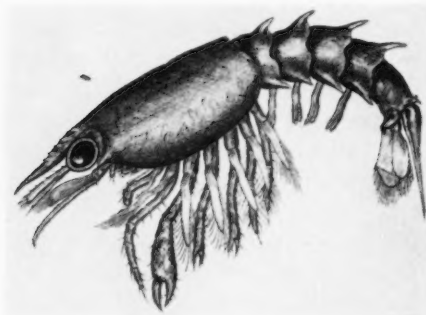


FIGURE 1.—Third-stage larval lobster, showing the position of the eyes and of the thoracic exopodites as they appear at the end of the downward stroke; also the abdominal swimmerets, which will become functional in the fourth stage.

was assumed to represent a reaction to the degree of light intensity in that area.

Since blackening the eyes of the larvæ seemed impracticable, the operation of blinding the young lobsters was performed by one of two methods,—either by searing the surface of the eye with a hot needle, or by excising the whole eye by cutting the eye stalk with a pair of small scissors. The former method gave, as a rule, better results; for, while “excising”

appeared to weaken the young lobsters, the “searing” seemed to disturb in no great measure their activity. In some groups of larvæ the left eye only was blinded, in other cases the right eye, and in still other cases both eyes. Such groups—or, in some instances, individual larvæ—were subsequently put in the rectangular glass container, which was placed in a large box blackened on the inside. This box was also fitted with windows of various sizes, and rested on the laboratory table in such a way that light was admitted to the glass container by means of such windows only. With this arrangement the aim was to ascertain either the index of reaction of groups of larvæ to the directive influence of the light, or to study the characteristic behavior of single blinded larvæ when subjected to similar light influences.

## II. REACTION OF NORMAL LOBSTERS.

About one hundred first-stage larvæ, from forty-eight to fifty-four hours old, were transferred from one of the large hatching-bags to a crystallization dish. When this was set within the dark box and submitted to light coming from one direction through the side window, every larva oriented with the head away from the light and swam backward toward the window side of the dish (Fig. 2).

When twenty of these larvæ were transferred to the glass-bottomed box, which was mounted over the light-shaft and overlying the colored glass plates, the reaction showed that the majority of larvæ grouped themselves in the most brightly illuminated area, as represented below:

Time.	Red.	Orange.	Green.	Blue.
3.10	0	1	1	18
3.15	1	2	4	13
3.20	0	0	2	18
3.25	1	0	2	17
Totals	2	3	9	66

Between each trial the position of the box was reversed. In the next instance the order of the glass plates was changed, and the results, which were as follows, show that the majority of larvæ still oriented in the brightest area of the box:

Time.	Green.	Red.	White.	Orange.
3.35	1	2	17	0
3.45	3	2	15	0
3.50	0	2	17	1
3.55	0	1	16	3
Totals	4	7	65	4

In the preceding lists it was observed that many of the larvæ which gathered over the white glass became oriented with the back downward, a position never favorable to progressive orientation. Since it was believed that this factor might be to some extent responsible for the gathering in the areas of the greatest illumination, in the next case the glass-bottomed box was set over a black background, the colored glass plates ranged in linear series over the top of the box, and the light admitted from above. The position of the glass plates was frequently changed, and the records of several tests, which were made at five-minute intervals, and which show that the larvæ still persisted in gathering in areas of greatest illumination, are as follows:

Red.	Orange.	Green.	White.
0	1	2	17
2	0	2	16
White.	Green.	Orange.	Red.
17	2	1	0
17	1	2	0
Green.	Orange.	White.	Red.
0	2	15	3
0	1	16	3

Experiments similar to the foregoing were conducted with fourth-stage and fifth-stage lobsters. Since the exopodites or thoracic appendages, by means of which the larval lobsters of the first three stages swim, are lost in the moult from the third to the fourth stage, some change in the type of reaction in the fourth and later stages might be expected. The differences which were found to exist are not of great importance to present considerations, but may be briefly outlined as follows: The normal fourth-stage lobsters, which for the greater part of the stage swim freely by means of the abdominal swimmerets, manifest throughout the stage-period a



negative phototactic reaction, which is accentuated towards the close of the stage. The photopathic reaction, which at the beginning is usually positive, gradually changes by the close of the stage-period to negative in the majority of fourth-stage lobsters. Generally speaking, the reactions of the fifth-stage lobsters are typical of the adult form, and are chiefly characterized by the light-shunning instinct. The phototactic and photopathic reactions are negative from the beginning of this stage to the end of it. As in the earlier stages, so in the fourth and fifth, the eyes play a most important rôle in determining the nature of the reaction to light, although the invariable tendency to "head" away from the light is never again, in the later life of the lobster, so strongly manifested as during the first three larval stages.

The results of the foregoing experiments show, first, that the normal first-stage larvæ react to the directive influence of the light rays by placing the longitudinal axis of the body, parallel to them, with the head away from the source of light, and by swimming toward that source. Lobsters in the later larval stages may at times, however, gather in the darker areas. Fourth-stage and fifth-stage lobsters do not manifest so strongly the tendency to place the axis of the body parallel to the direction of the incident light rays, although they do undergo definite progressive orientations.

### III. REACTIONS OF LOBSTERS WITH ONE EYE BLINDED.

From the group of first-stage larvæ which, as has been shown above, were reacting positively to light, ten were selected and blinded, by means of a hot needle, in the left eye. Subsequently, these ten larvæ were put in the glass container, and this placed in the dark box. When the light was admitted through a small window to the side of the glass container, the larvæ underwent both a body-orientation and a progressive orientation. Both these orientations, however, usually occurred only when the intensity of light was not great, and both differed in their nature from the reaction of normal larvæ under similar conditions. The body-orientation was not characterized by placing the longitudinal axis of the body exactly parallel, but slightly oblique, to the direction of the light rays, as shown in Fig. 3; the progressive orientation took place as in normal larvæ, except that the reaction was less definite. The latter may be outlined as follows:

Positive.	Neutral.	Negative.
7	3	0
6	3	1
4	4	2
3	5	2
7	2	1
4	3	3
31	20	9

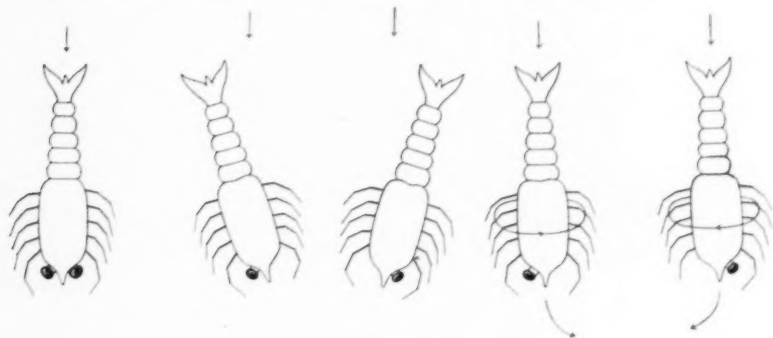
Next, these same larvæ were transferred to the glass-bottomed box mounted on the colored glass plates over the light-shaft. Records of the position of the larvæ were made at five-minute intervals, between which the box was reversed, and resulted as follows:

Red.	Orange.	Green.	White.
2	2	1	5
1	2	2	5
0	0	4	6
0	0	2	8
3	4	9	24

Similar experiments to ascertain the effect of blinding one eye upon the definiteness of reaction of fourth-stage and fifth-stage lobsters gave much the same results as in the case of the larvæ. The index of response was the same as that of normal lobsters from the same group, but the small number which at any one time would give a definite reaction, either positive or negative, showed that blinding had seriously disturbed the functions of some organs through which orientation to light is brought about.

These general results depend upon a large number of experiments on lobsters in all stages of development. Only a sufficient number of instances can be here reported to give a general idea of the reactions.

**The methods of reaction.**—There remains to be mentioned the method of body-orientation in larvæ having one eye blinded. The writer has already called attention<sup>4</sup> to the fact that, in the case of normal larvæ, the behavior from one minute to another is made up of a large number of strange movements, — rotations, revolutions, swingings, turnings; furthermore, that these peculiar gymnastics are dependent upon the relative intensity of the light striking the eyes of the larvæ: light from one side determines a quick turning in the opposite direction; light from above precipitates



FIGURES 2-6.

the larva head first, or at least causes the assumption of a new swimming-position with the head downward; while light from below causes the larva to assume a body-position with the head directed slightly upward.

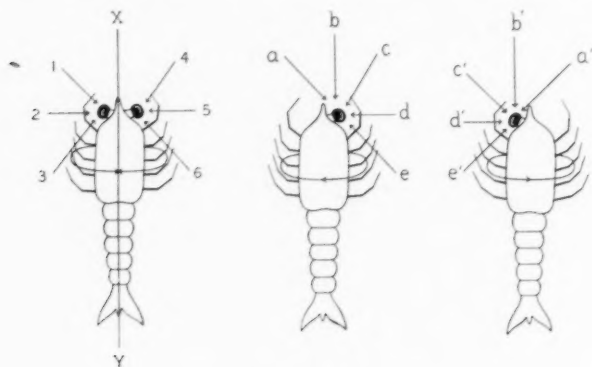
In the case of larvæ with the left eye blinded the results were found to be quite different. The swingings and rotations were invariably in a definite direction. Very frequently the most common reaction directly following the removal or blinding of the left eye was a long series of rotations on the longitudinal axis of the body. These rotations occurred with great rapidity, often at the rate of 150 per minute, and took place, moreover, in a determined direction, as shown in Figs. 5 and 6. Usually these rapid rotations soon subsided, although they might for an hour or more re-occur at intervals. When the larvæ attempted forward swimming, the line of progress invariably became an arc curving toward the side of

<sup>4</sup> HADLEY: Annual report of the Rhode Island Commission of Inland Fisheries for 1906.

the injured eye, as shown in Figs. 5 and 6, and often the larvæ swam for several moments in small circles, about 3 c.c. in diameter.

If the right eyes of the larvæ were blinded, the resulting reaction was reversed. The body rotations were now in the direction indicated in Fig. 6, while in the case of straightforward swimming the progression took place in the direction of an arc curving as shown in Fig. 6.

It is not difficult to find the reason for these types of reaction in larvæ with blinded eyes. Any rotation of the larvæ on the longitu-



FIGURES 7-9.

dinal axis of the body is caused by unequal forces exerted by the thoracic exopodites on opposite sides of the body. In instances where rapid and continued rotations occurred, the vibration of the exopodites on the side of the blinded eye was reduced to a minimum, while in some cases the stroke of the exopodites on the side of the injured eye appeared to be correspondingly accelerated. Under natural conditions of eye stimulation the forces exerted by the exopodites on opposite sides of the body exactly counterbalanced one another. But when, for any reason, one set beat more rapidly than the other, there resulted either a single rotation, or a series of rotations on the long body axis, or a swing of the long axis toward the right or toward the left, according as the greater intensity of light was brought to bear on the right or the left eye of the larva.

In this connection one other point regarding the direction of light striking the injured eye and the localization of the stimulus is of

interest. If the light was introduced to a normal larva from direction designated 1 (Fig. 7), the head of the larva would swing toward the right; while, if the light was introduced in the direction 6, the head of the larva would swing toward the left. The reason for this is that the greater intensity of light on one eye causes a stronger action of the exopodites on that side, with a consequent interruption of the equilibrium, and the larva swings toward the opposite side, or, under certain conditions, undergoes a rotation on the longitudinal body axis. In other words, the direction of turning of the larva is invariably determined by the direction from which the light comes, and the consequent inequality of illumination of the two eyes. It is, therefore, easy to understand why, in the case of the normal larvæ, the turn is always in the direction of the shortest path by which the head may come to face directly away from the source of light.

Here, again, is a great difference when we attempt to contrast with the above-mentioned type of reaction that of larvæ with a blinded eye. When the left eye was blinded and the light was allowed to strike the uninjured right eye, the reaction was the same, from whatever direction the light might come. If, for instance, the larva was represented as in Fig. 8, and the light entered from the direction designated by arrows *a, b, c, d, e*, upon the right uninjured eye, the direction of movement was toward the left in every case; or if rotations on the longitudinal axis took place, these were invariably in the direction represented by the arrow passing around the body of the larva, as in Fig. 8.

If, on the other hand, the right eye was blinded and the rays of light came from the direction represented by the arrows in Fig. 9, then the turning of the larva was toward the right; or if rotations on the longitudinal axis occurred, these rotations were in the direction indicated by the arrow passing around the body of the larva, as in Fig. 9.

It thus becomes clear that the mechanics of orientation in the case of larvæ with one injured eye does not involve a swinging which brings the body by the shortest path to lie parallel to the direction of the light rays, as is the case for larvæ with normal eyes. The typical reaction is a swinging in a single direction; yet this direction may not be, at first, away from the source of light, but even more fully toward it than before. This point is represented more clearly by Figs. 7, 8, and 9. Let Fig. 7 represent a

larva with both eyes normal, Fig. 8 one with the left eye blinded, and Fig. 9 one with the right eye blinded. When, now, the light coming in the direction of the arrow 1, Fig. 7, struck the eyes of the larva, the reaction was an immediate swing toward the right, so that the long axis ( $x - y$ ) of the larva came to lie parallel to the direction of the incident light rays, and the head pointed as represented by arrow 1. If, again, the rays impinged in direction of arrow 4, then the swing was toward the left and the longitudinal axis of the body assumed the direction of the incident rays, and with the head pointing as shown by arrow 4. The same method of reaction obtained in the orientation to light coming from the directions indicated by the arrows 2, 3, 5, 6. In all these instances the swing of the larva was by the shortest path that would bring the head to face away from the source of light, and the longitudinal axis of the body to lie parallel to the direction of the incident rays.

In Fig. 8 the conditions represented were different. When the light rays came from the direction indicated by the arrow *a*, the direction of swinging of the body-axis was invariably the same; the larva would swing to the left and through the supplement of the angle, through which it naturally would swing if both eyes were normal and the light came from the same direction. This method of orientation invariably took place, notwithstanding that in this swing the head of the larva must face directly, though momentarily, the source of light,—a condition of affairs quite unknown in the behavior of normal lobster larvæ. Here there are no conditions known that can cause a larva to face the source of light, even for an instant. Every movement serves to one end,—to bring the head away from the source of illumination. Yet, under the conditions represented in Fig. 8, if the light came from any of the directions indicated by arrows *c*, *d*, *e*, the resultant reaction was the same as that in the case of larvæ with both eyes normal.

In the conditions indicated by Fig. 9 the principle of the reaction was the same, but the results were reversed. In this case the right eye was blinded. If now the incident light rays took the direction represented by arrow *a'*, the larva would swing, not toward the left (as in the instance of normal larvæ, Fig. 7), but toward the right. In other words, the longitudinal axis of the larva would pass through the supplement of that angle through which it would naturally swing if both eyes were normal and the light came from the same direction. In case, however, the light came from the

direction designated by the arrows  $c'$ ,  $d'$ ,  $e'$ , then the reaction is the same as that of larvæ with both eyes normal. In other words, when in both the last cases (illustrated by Figs. 8 and 9) the light struck the larvæ from the side of the normal eye (Fig. 8  $c$ ,  $d$ ,  $e$ ; Fig. 9  $c'$ ,  $d'$ ,  $e'$ ), then the resulting reaction was similar to that which occurred in larvæ with uninjured eyes: the larva would swing directly away from the light by the shortest path that would bring the uninjured eye to face away from the source of light (Figs. 2 and 3). Yet in this instance the long axis of the body was never brought exactly parallel to the direction of the light rays, but at a slight angle to them, so that the uninjured eye received the least possible stimulation. But even this body-orientation was seldom long maintained, since the tendency to manifest circus movements or to progress in an arc curving toward the side of the injured eye was ever present, regardless of the direction of the light rays. It is presumable that, for this reason, when larvæ with one eye blinded were placed under the influence of light from different directions, they might be observed to invariably progress by swingings or by rotations toward the side of the blinded eye. This aspect of behavior is to be seen in blinded larvæ of all the early stages, but most definitely during the first-stage period.

Observations on the behavior of fourth-stage and fifth-stage lobsters having one eye blinded gave similar results, although the tendency to assume body-positions with the head directed away from the light was not so prominent in these and later stages. In the fourth-stage lobsters with one eye blinded, the definiteness of both photopathic and phototactic response was lessened. The effect of illumination upon the good eye, moreover, was similar to that obtained in larvæ in earlier stages, except as it was modified by the difference in the manner of swimming which obtains between lobsters of the fourth and earlier stages. The free swimming of the fourth-stage lobsters having one blinded eye was invariably in an arc which curved away from the side of the uninjured eye. But while in the first-stage larvæ this arc of turning was usually one of small radius, the swimming-curve of the fourth-stage lobsters was one of much greater latitude; so that, for instance, when such lobsters were confined in a crystallization dish having a diameter of 150 mm., they swam in a circle about the rim of the dish and always in the same direction: if the right eye was blinded, clockwise; if the left eye was blinded, counter-clockwise. Often the



operation of blinding acted as a temporary stimulus to more energetic swimming. In such cases the larva would swim actively for a brief time in a circle of large radius. As fatigue increased, the radius of the circle decreased, with the result that a spiral-like course of progression was generally produced. The amplitude of the spiral gradually diminished, and eventually the lobsters would come to rest and fall to the bottom of the dish. It may here be noted that the rotations on the longitudinal body-axis, characteristic of larvæ in the first three stages blinded in one eye, never occurred in lobsters of the fourth or later stages. This is of course determined by the loss of the thoracic exopodites, by which in the early stages this rotary motion is produced.

In the fifth-stage lobsters the tendency to crawl over the bottom rather than to swim at the surface modified to no great extent the nature of the reaction in individuals having one eye blinded. Here also was manifested the same tendency to crawl in a circle and always away from the side of the good eye, and with the injured eye looking toward the centre of the circle.

#### IV. REACTIONS OF LARVÆ WITH BOTH EYES BLINDED.

From the same large group of larvæ from which the blinded lobsters mentioned in the last section were taken, ten others were selected, and their reactions tested in the rectangular container and in the glass-bottomed box. In both instances a definite positive

Positive.	Neutral.	Negative.
2	5	3
3	4	3
4	3	3
5	5	0
14	17	9

reaction resulted, and the mechanics of orientation was found to be similar to that observed in all previous cases.

Next, these larvæ were blinded, by means of the hot needle in both eyes. After the operations it was observed that blinding the

second eye, although it diminished the activity, appeared rather to re-establish the equilibrium than to cause a complete cessation of swimming movements in both the right and left sets of thoracic exopodites. When these fully blinded larvæ were transferred to the rectangular compartment, and this placed in the dark box, the reaction was as recorded in the preceding table.

Subsequently these larvæ were placed in the glass-bottomed box over the light-shaft, and submitted to the influence of the monochromatic lights. Many tests were made at five-minute intervals, and the position of the box was reversed between each test. The following record is characteristic of all:

Red.	Orange.	Green.	White.
3	2	2	3
2	2	2	4
2	1	3	4
3	2	4	1
10	7	11	12

When these larvæ were submitted to the influence of lights coming from many different directions, as in a room illuminated by several windows, no particular body orientation to the light could be observed, and the mechanics of orientation involved a turning, now in one direction, now in another. In other words, the reactions determined by the conditions of light were at a minimum,—as much so as the reactions of larvæ in deep twilight or darkness.

These records are typical of the results of many tests, the details of which cannot now be presented. In sum, they appear to demonstrate that the larval lobsters blinded in both eyes underwent no definite orientation to light, but became scattered homogeneously through the area of their confinement. Similar results, moreover, were obtained with fourth-stage and fifth-stage lobsters. Here also no definitely positive or negative phototactic or photopathic reaction was observed, but a homogeneous scattering, such as took place in the case of the larval lobsters.

## V. CONCLUSIONS.

Perhaps the most important issue to which the foregoing observations lead involves, primarily, a consideration of the very intimate connection between the optical stimulation and the definite muscular reactions, which in the larval lobsters invariably follow. And co-ordinate with this point is the question of the degree to which the method of "trial and error" may enter into the behavior of the young lobsters, normal or blinded.

In the light of the experiments which have been recorded, it appears that stimulating with light one eye of a young lobster has its effect—depending upon the stage of the larva—upon three different sets of appendages which serve the purpose of locomotion; and that such stimulation is effective only on that side of the body which corresponds with the uninjured eye. In the first three larval stages the means of locomotion are the exopodites of the thoracic appendages. In the fourth stage, which is the free swimming stage *par excellence* of the lobster's existence, the means of swimming is found in the swimmerets, the paired, paddle-like abdominal appendages; while in the fifth-stage lobster, which is essentially a crawling animal, the usual means of progression is limited to the thoracic ambulatory appendages,—the legs proper, although in this stage also there may be brief and infrequent periods of swimming. In each of these cases it has been demonstrated that a very intimate connection exists between the eye, on one hand, and, on the other, the exopodites, the swimmerets, and the legs proper, on the same side of the body. A slight change in the intensity of light striking one eye is immediately followed by a succession of muscular extensions and contractions which concern alone that side of the body, and which determine, through medium of the nervous system and the appropriate appendages, the immediate assumption of a new body-position, which may be one of rest or one of continued movement.

Nothing can be farther removed from the orienting reaction of the larval lobsters to light than the method of "trial and error." The larval lobsters are, to all practical purposes, mere machines, upon which the forces of light act through the medium of the eyes, nervous system and appendages, and produce a constant and invariable result. There is no random movement, no evidence of "choice." The turning of the larval lobster in a certain direction

is dependent upon the vibration of the exopodites of the thoracic appendages; the vibration of the exopodites is dependent upon nervous excitation; this excitation is produced and regulated by the stimulation of the eyes of the larva. If one side of the larva receives more light than the other side, the eye on that side receives more stimulation. In consequence, the exopodites on that side beat the water more strongly and rapidly, and the larva "comes around" like a skiff to a position in which the equal stimulation of the two eyes determines an equally strong vibration of the exopodites on each side of the body, and consequently the re-establishment of a balanced equilibrium. In this instance the light impinging upon the eyes of the larva regulates the activity of the exopodites as regularly and as definitely as an electric current the movement of a magnet bar. That the movement of the exopodites causes the larva to head away from the light is not difficult to explain. The centre of the force exerted by the exopodites is anterior to the centre of equilibrium of the larva. When one eye is directed more nearly toward the light, the force of the vibrating exopodites is so applied as to swing the anterior end of the body. As soon as this end has assumed such a position that the eyes are no longer stimulated, or equally so, then the movements of the exopodites cease, or the vibration of the right and left sets counterbalance each other.

When one eye is blinded, the stimulus needful to maintain the vibratory movements of the exopodites on that side of the body is absent, and, in consequence, the anterior or head end of the larva is swung out of line as a result of the stronger action of the exopodites on the opposite side, whose activity is still sustained by stimulation through medium of the uninjured eye, until, by the turning of the larva, this eye comes to lie in the shadow. When both eyes are injured, the equilibrium has a tendency to become re-established by reason of an equal lessening of stimulus on each eye. That, under these conditions, some other factor than stimulation by light is operative in maintaining the vibration of the exopodites is clear, for these swimming movements, although much diminished in rapidity and force, still keep the larva in a kind of equilibrium, minus either body-orientation or progressive orientation. This continued action of the swimming appendages may be attributed to imperfect blinding, but more likely to mechanical irritation resulting from the operation of searing the eyes or of cutting the eye stalks.

The point of concise localization of function of different areas

of the relatively large compound eyes, as investigated by Holmes,<sup>5</sup> was not undertaken in connection with the present work. Holmes ascertained that if only the lateral surface of one eye of *Ranatra* was exposed to light, the action of the flexor muscles on the same side, and of the extensor muscles on the opposite side, of the body was increased. The tendency to produce circus movements was checked and followed by a turn in the opposite direction. Holmes attributed this turn to the diminished amount of light received by the eye as it turned away from the source. This diminution, he concluded, acted as "a stimulus to a movement in the opposite direction." In the larval lobsters the lighted area of the eyes was never mechanically restricted, and it is not known what effect such limitation of the surface illuminated would have had upon the behavior. It was clear, however, that in the lobster larvæ with one eye fully blinded there was no such conflict of impulses as described by Holmes.<sup>6</sup> No other tendency, nor any influences, save that of diminishing the light, hindered the continuance of the circus movements; and even in the latter case there was no inclination to turn in the opposite direction, but merely a tendency to remain unresponsive in the same body-orientation. Regarding the mechanics of this reaction, moreover, the stimulation of one eye by light was not observed to cause an increase in the action of the flexor muscles on the same side and of the extensors on the opposite side. Such stimulation increased the action of *both* the flexors and extensors on the same side, whether these muscles were associated with the thoracic exopodites, the abdominal swimmerets, or the legs proper; moreover, both phases of muscular action in the corresponding appendages of the opposite side of the body were proportionately diminished.

Although those results correspond, in a degree, with those obtained by Holmes<sup>7</sup> from experiments with blinded amphipods, certain exceptions are to be noted. His general conclusion was as follows: "Blackening over one eye of the terrestrial amphipods and in several positively phototactic species of insects causes the animal to perform circus movements with the unblackened eye looking toward the centre of the circle. Blackening over one eye in negatively phototactic amphipods causes circus movements to be

<sup>5</sup> HOLMES: *Journal of neurology and psychology*, 1905, xiv, p. 305.

<sup>6</sup> HOLMES: *Loc. cit.*

<sup>7</sup> HOLMES: *This journal*, 1901, v, p. 211.

performed in the opposite direction." This statement of the case indicates that in positive animals the light acts as an inhibiting agent producing lesser movement on the side of the body upon which it acts through the medium of the unblackened eye, while in negatively phototactic animals, on the other hand, the light acts as a stimulating agent on that side of the body which it influences through the medium of the unblackened eye.

In the lobster larvæ all the progressive reactions which took place immediately following the blinding of one eye were positive. In certain cases it appeared that either the operation itself or the effects of blinding changed the index of reaction from negative to positive. In all these instances, whether the previous reaction had been negative or positive, the resulting behavior was the same: a series of revolutions, of circus movements, or a progression in which the direction of turning indicated that the influence of light on the unblackened eye was to cause greater activity of the swimming appendages on that side of the body, while blinding invariably had the opposite effect. In other words, the reaction of the blinded positively reacting lobster larvæ corresponds with those of Holmes's negatively reacting amphipod, *Hyaella dentata* (Smith), but not with his positively reacting amphipods.

The reactions which have been described in the foregoing pages were characteristic of the larval lobsters, whether they were, at the time or previously, reacting positively or negatively to light. As the writer has shown in other papers,<sup>8</sup> the index of the phototactic response depends upon the intensity of the light which strikes in equal degree the two eyes of the larva, and determines whether the exopodites shall beat forward and downward or backward and downward. This difference in stroke (especially the "posterior" or "negative position") is not manifested to so great an extent in larvæ with one blinded eye, and it is perhaps for this reason that such larvæ manifest so slightly the definitely negative progressive orientation. The writer hopes at a later date to study more fully the method employed by blinded larvæ in their continuous progression toward the light or away from it. It has been the aim of the present paper merely to give the general results of such reactions, and to describe, more in detail, the immediate changes induced by light upon the body-orientation with respect to the direction of the light rays.

<sup>8</sup> HADLEY: *Loc. cit.*; also, *This journal*, 1906, xvii, p. 326.

## VI. SUMMARY.

1. **Reaction of normal larvæ**—(a) The normal first-stage larvæ, within forty-eight to sixty hours after hatching, react to the directive influence of the light rays by placing the longitudinal axis of the body parallel to the incident rays, and then, with the head directed away, approaching their source. After the second day negative reactions may be manifested.

(b) If submitted to the influence of non-directive light, the larvæ usually congregate in the areas of brightest illumination. These reactions are definite and strongly manifested.

(c) The mechanics of orientation involves direct motor reflexes, due to the unequal stimulation of the two eyes, and accomplished through medium of the nervous system and thoracic exopodites, whose action causes the larvæ to place their longitudinal axes parallel to the direction of the light rays. In the fourth stage the swimming is accomplished by the abdominal swimmerets, while the fifth-stage lobster crawls with the true legs.

2. **Reaction of larvæ blinded in one eye.**—(a) The larvæ of any of the first three stages, when one eye is blinded, react to the directive influence of the light and to differences in intensity like normal larvæ, except that the reactions are much less definite, and are seldom negative except in fourth and later-stage lobsters.

(b) The immediate results following the destruction of photo-reception in one eye are: (1) The production of rapid rotations (often at the rate of 150 per minute) on the longitudinal axis of the body, which are invariably in a determined direction,—that shown in Figs. 5 and 6; (2) A type of progression in which the larva continually performs "circus movements," or turns toward the side of the injured eye.

(c) The mechanics of orientation in such cases involves direct motor reflexes dependent upon the over-stimulation of one set of thoracic exopodites through the medium of the uninjured eye, and the lack of stimulation of the opposite set of exopodites as a result of destroying the photo-receptors in the blinded eye.

3. **Reaction of larvæ blinded in both eyes.**—When both eyes of the larvæ are blinded, there is no reaction, either to the directive influence, or to differences in the intensity, of light. In such cases the progressive swimming is weak, but more balanced than in larvæ



with one blinded eye, since the photo-receptivity in both eyes is the same.

4. The reactions of blinded fourth-stage and fifth-stage lobsters correspond to those of the larval lobsters, except as the former are modified by the swimming and crawling methods of progression respectively characteristic of lobsters in the fourth and fifth stages.

5. The direction, *per se*, of the light rays (unless it be because of a certain course through the body) has no influence in bringing about a reaction: If approximately the same area of the right eye is stimulated by light coming from any direction, the resulting reaction is the same.

6. The evidence gained from observation on the behavior of blinded and of normal lobsters demonstrates that many of the complexities of behavior (especially in the body-orientations) in the larval and early adolescent lobsters can be reduced to, and explained on the basis of, simple motor reflexes, which show no trace of random movement or of "choice" and have nothing in common with the method of "trial and error."

THE RELATION OF IONS TO CONTRACTILE PROCESSES. — II. THE RÔLE OF CALCIUM SALTS IN THE MECHANICAL INHIBITION OF THE CTENOPHORE SWIMMING-PLATE.

By R. S. LILLIE.

[*From the Marine Biological Laboratory, Woods Hole, and the Laboratory of Physiological Zoölogy, University of Pennsylvania.*]

IN a former paper<sup>1</sup> I referred briefly to a special and somewhat unusual peculiarity of the Ctenophore swimming-plate, namely, its susceptibility to inhibition through slight mechanical stimuli in sea water and certain artificial media containing calcium: "Slight stimulation of a row of beating plates, a detached portion of a row, or even an active individual plate, with the extremity of a glass rod, is typically followed by immediate and complete cessation of movement. After an interval activity is resumed." This arrest of activity is extremely characteristic of normally beating plates, and as a rule instantly follows jarring or shaking the vessel containing the animals or portions of the rows of plates. It is independent (though always an accompaniment) of another effect which — especially in the more muscular forms, as *Beroë* — also results from mechanical stimulation of the rows, namely, a withdrawal of the latter below the general surface of the animal through a contraction of radially disposed muscle fibres. In *Beroë* this contraction is so vigorous and persistent as to render this animal quite unsuitable for the following kind of experimentation; but in *Eucharis* and *Mnemiopsis* — forms with relatively weak musculature — the retraction is slight and temporary; strips containing plates cut from either of these animals relax almost immediately, and the plates remain freely exposed to the sea water as normally. After a more or less prolonged period of quiescence, due to the operation and lasting from a few seconds to a minute or more, the strips resume activity, the waves of move-

<sup>1</sup> R. LILLIE: This journal, 1906, xvi, p. 117. My obligations are due to the Carnegie Institution for aid in a portion of the present research.

ment running intermittently along the rows from aboral to oral in the typical manner. If such a beating row of plates is jarred or shaken or touched with a glass rod, the movement instantly ceases; after a variable interval, usually not lasting for more than a few seconds, the plates, if undisturbed, resume beating. The arrest of movement may be localized by gently touching the plates at the oral end of a row; these then cease beating, while those more aborally situated may remain active. If the more aboral plates are thus inhibited, the whole row usually becomes quiet. In these forms the impulse causing successive members of the row to beat always travels from aboral to oral, even in detached portions of the animal; and the centrally and orally situated plates of a detached strip seem usually to depend for their stimulation on impulses initiated in the active plates at the aboral extremity and thence transmitted along the row. These plates thus determine the rhythm of the entire row. Each plate is, however, capable of independent movement, although, as a rule, the "metachronous" characteristic, *i. e.*, the wavelike successiveness of the movement, is apparent, indicating an interdependence of the above kind.

The susceptibility to this form of mechanical inhibition shows a certain variability with the condition of the tissue. In specimens of *Eucharis* or *Mnemiopsis* — or in portions of swimming rows from these animals — kept in dishes for one or two days the movement undergoes by degrees an alteration in character, becoming more continuous and unintermittent as well as more rapid in rhythm; this change is accompanied by a decreasing susceptibility to mechanical inhibition, and the arrest of movement may finally be only momentary, even with a stronger stimulus than before. This alteration in response seems to be in some way dependent on the progress of conditions favoring coagulative changes in the contractile substance, since it is especially evident in plates whose substance is beginning to lose its normal clear transparency; this change is shortly afterwards succeeded by the definite whitening or coagulation that always in these animals precedes the death and disintegration of the plates.

It is only when conditions are normal or approaching the normal that mechanical stimulation inhibits contractile activity. It is not difficult experimentally to induce in the plates a state in which mechanical stimulation has an effect precisely the reverse of this — exciting movement in inactive plates and stimulating rather than repressing movement in those already active. The abnormally accel-

erated vibratory movement observed in pure and especially in weakly acidulated solutions of alkali or alkali earth salts<sup>2</sup> is absolutely insusceptible to mechanical inhibition; and plates that fail at first to vibrate in such solutions — as not infrequently happens — may readily be stimulated to energetic activity by poking or stirring with a needle or a glass rod. Mechanical stimulation thus produces under these conditions an effect directly opposite to that observed normally; the absolute lack of any inhibition through mechanical influence, once vibration has begun in these solutions, is in striking contrast to the behavior in the normal medium.

The inhibitory effect is thus dependent on a certain constitution of the surrounding medium. In media of composition similar to that of sea water the calcium salts play an especially important rôle. If these are removed, the plates are found after an interval to be no longer subject to mechanical inhibition. The dependence of inhibition on the presence of calcium salts is indicated by the following experiment:

Portions of the ciliated rows of *Eucharis multicornis*<sup>3</sup> were placed in an artificial calcium-free sea water of the following composition: 30 gm. NaCl, 6.6 gm.  $\text{MgSO}_4$ , 0.8 gm. KCl in 1000 c.c. of solution.<sup>4</sup> After an interval of one to two minutes typical movements begin, and in five minutes all the plates are beating rapidly and regularly with a rhythm more rapid than in normal sea water. This movement is not noticeably checked by jarring or by pulling the portions of tissue through the solution with forceps, but continues undisturbed in active regular rhythm.

In *Mnemiopsis*, so far as I have observed, the conditions of activity are essentially the same as in *Eucharis*, and in the majority of the experiments on the relation of salts to mechanical inhibition I have used the common Woods Hole species, *Mnemiopsis leidyi*. The following experiment is typical:

September 9, 1906. — A strip from a row of active plates of a *Mnemiopsis* was placed at 11.30 A. M. in the following van't Hoff's solution: 100 volumes  $m/2$  NaCl + 2.2 vols.  $m/2$  KCl + 7.8 vols.  $m/2$   $\text{MgCl}_2$  + 3.8 vols.  $m/2$   $\text{MgSO}_4$ . The plates, after remaining inactive at first for a few minutes, resume active regular beating movement. At first the movement may be

<sup>2</sup> Cf. my former paper, already cited, pp. 124 seq.

<sup>3</sup> The species chiefly used in my experiments at Naples; called by an inadvertence *Eucharis lobata* in my former paper.

<sup>4</sup> HERBST: Archiv für Entwicklungsmechanik, 1900, ix, p. 424.

inhibited, though not readily, by mechanical treatment; later the plates lose all susceptibility, vigorous stirring and pulling through the solution having no apparent influence on their activity. Occasionally the plates, if left to themselves, may cease movement spontaneously; they are then easily stimulated mechanically to renewed activity. After twenty-two hours in the solution activity still remains, though the beats are somewhat feeble; mechanical treatment has no inhibitory action, but, on the contrary, augments the movement; at this time the plates are clouded and whitish in appearance, indicating incipient coagulation.

In the control experiment—a strip from the same animal in sea water—slight mechanical stimulation due to contact of a glass rod, or even shaking or jarring the dish, promptly arrests movement at any time during September 9 up to 4.15 P. M. The next morning, after twenty-two hours, the plates are also readily inhibited in this manner, but activity is resumed sooner and is less intermittent and of a more rapid rhythm than before. Several plates at one end of the strip are only slightly sensitive to mechanical inhibition (this decrease in susceptibility in portions of rows kept for some time in sea water is typical).

The following experiments illustrate the effect of addition of small quantities of a calcium salt to the van't Hoff's solution:

Strips containing rows of plates from a large *Mnemiopsis* were added to the following solutions at 3.45–3.50 P. M., September 10, 1906.

1. 100 vols. van't Hoff's solution ( $m/2$ ), without Ca (*control*).
2. 100 vols. van't Hoff's solution ( $m/2$ ), +  $\frac{1}{2}$  vol.  $m/2$   $\text{CaCl}_2$ .
3. 100 vols. van't Hoff's solution ( $m/2$ ), + 1 vol.  $m/2$   $\text{CaCl}_2$ .
4. 100 vols. van't Hoff's solution ( $m/2$ ), + 2 vols.  $m/2$   $\text{CaCl}_2$ .
5. 100 vols. van't Hoff's solution ( $m/2$ ), + 4 vols.  $m/2$   $\text{CaCl}_2$ .
6. 100 vols. van't Hoff's solution ( $m/2$ ), + 8 vols.  $m/2$   $\text{CaCl}_2$ .

The action of these solutions was as follows:

1. (*Control*.) At 4.30 P. M. the plates are in active movement which cannot be inhibited mechanically; plates spontaneously inactive are stimulated to renewed activity by contact with the glass rod. Next day (after seventeen hours) plates are partly clouded, and beat somewhat weakly; the glass rod stimulates slightly; no inhibition.
2. At 4.30 P. M. the plates are beating actively; mechanical inhibition is absent or slight; mechanical treatment often stimulates. Next day (seventeen hours) conditions are similar to these in the control.
3. 4.30 P. M. In general similar to Solution 2, but the movement is more intermittent and shows slight inhibition. Next day (after seventeen hours)

the plates are active and unclouded ; movement is stimulated rather than inhibited by contact with the glass rod.

4. At 4.30 P. M. the plates are beating actively and continuously ; the movement is readily inhibited by contact or jarring. Next day (after seventeen hours) movement is active and intermittent, travelling in irregular waves along the row ; the plates remain transparent. Mechanical inhibition remains, though less marked than yesterday, and movement is quickly resumed ; after twenty-three hours inhibition is slight or absent ; movement is active, and the plates are slightly clouded.
5. 4.30 P. M. The plates remain for the most part inactive with only occasional spontaneous beats. Next day (seventeen hours) there is some intermittent activity, very easily inhibited ; a slight touch invariably inhibits the active plates and has practically no effect on inactive.
6. At 4.30 P. M. the plates, though normal in appearance, remain persistently inactive ; no spontaneous movement is seen, and mechanical treatment fails to stimulate. After seventeen hours all are coagulated and inactive.

The use of  $m/2$   $\text{SrCl}_2$  in place of  $m/2$   $\text{CaCl}_2$  in Solutions 2 and 3 (further addition of  $\text{SrCl}_2$  precipitates  $\text{SrSO}_4$ ) produces no evident effect. The addition of  $\text{SrCl}_2$  is here insufficient for purposes of comparison ; it will be seen later that strontium can replace calcium only to a limited degree.

From the above experiments it appears that an increase in the proportion of calcium in the medium renders the plates more and more sensitive to the inhibitory influence of contact or shaking, and decreases the susceptibility to stimulation by the same means. The spontaneous activity of the plates is also diminished in the presence of calcium ; it is slight in Solution 5 (with 4 volumes  $m/2$   $\text{CaCl}_2$ ) and completely absent in Solution 6. The intermittence of the normal movement in sea water thus appears to depend on the presence of calcium, — *i. e.*, spontaneously acting internal influences tending to check the movement are either absent or ineffective when the medium is deficient in calcium. In general, therefore, increase in calcium facilitates processes tending to inhibition of the contractile activity, while decrease has the opposite effect. The calcium ions in the tissue for some reason exercise a repressive influence on the contractile process.

A repetition of the above experiments with a van't Hoff's solution in which magnesium sulphate was omitted and replaced by magnesium chloride gave an almost identical result. The sulphate ion is thus unimportant in relation to inhibition.

The following solutions were employed: Solution 1: van't Hoff's solution without sulphate (100 vols.  $m/2$  NaCl + 2.2 vols.  $m/2$  KCl + 11.6 vols.  $m/2$   $MgCl_2$ ); Solutions 2-6: the same with the addition, respectively, of  $\frac{1}{2}$ , 1, 2, 4, and 8 vols.  $m/2$   $CaCl_2$ . Strips with plates from an active *Mnemiopsis* were added to the solutions at 3.45 to 3.50 P.M., September 10, 1906. The results were as follows:

1. *Control* (van't Hoff's solution —  $SO_4$ ). An hour after placing in solution the plates show active and continuous movement which is *stimulated* and not inhibited by contact with a glass rod. Next morning (after eighteen hours) the plates are somewhat clouded; the movement is stimulated by contact; no inhibition.
2. 100 vols. solution +  $\frac{1}{2}$  vol.  $m/2$   $CaCl_2$ . After an hour movement is active and there is practically no inhibition; usually the glass rod stimulates. After eighteen hours conditions are as in Solution 1.
3. 100 vols. solution + 1 vol.  $m/2$   $CaCl_2$ . After an hour movement is active and shows only slight and inconstant inhibition; after eighteen hours the plates are transparent and vigorously active; mechanical inhibition is either evanescent or absent; the glass rod stimulates. A few hours later (twenty-three hours) most of the plates are in active movement and show slight clouding; no inhibition. All are coagulated next morning.
4. 100 vols. solution + 2 vols.  $m/2$   $CaCl_2$ . After an hour the plates are largely inactive or show intermittent movement; contact with the rod always inhibits movement and does not stimulate inactive plates. Next morning (eighteen hours) the plates are transparent and show vigorous intermittent movement; mechanical inhibition is well marked, but the movement tends soon to be resumed. After twenty-three hours the plates are less sensitive to inhibition. After forty-two hours many plates show active vibratory movement, and susceptibility to inhibition is slight, though still apparent; at this time the plates are beginning to coagulate.
5. 100 vols. solution + 4 vols.  $m/2$   $CaCl_2$ . After an hour the plates show almost no spontaneous activity. Next morning (eighteen hours) the movement is intermittent, and the plates remain inactive for the greater part of the time; the movement itself is active and easily inhibited; plates are transparent or slightly clouded. At twenty-three hours the plates have begun to coagulate; the living plates still show intermittent activity; inhibition is present, though less pronounced than before. Next morning all the plates are coagulated.
6. 100 vols. solution + 8 vols.  $m/2$   $CaCl_2$ . The plates show no spontaneous movement at first and cannot be stimulated. Next morning (eighteen hours) most plates are coagulated; a few are living and intermittently active; these are easily inhibited. A few *partly coagulated* plates show vibratory movement; this is not easily inhibited.



The general result is thus identical with that of the preceding series of experiments. The omission of the sulphate has no evident influence. Certain relations appear with great constancy in this and similar series. First: spontaneous movement, which ordinarily is arrested by the mechanical treatment accompanying the transfer of the strips to the solutions, is resumed in the solution after an interval. This interval is brief in the solutions containing no calcium; in the others it is found in general to be more prolonged the larger the proportion of calcium, and in solutions of relatively high calcium content several hours may elapse before spontaneous contractions begin. Second: plates in solutions containing excess of calcium (as Solutions 5 and 6 above), while at first perfectly quiescent, later become active. This activity is at first intermittent and very easily inhibited; later it becomes more continuous, and inhibition is more difficult and less lasting; still later, especially when the plate substance has become clouded, the vibrations become both more rapid and more continuous, and are frequently not inhibited, but on the contrary stimulated, by mechanical treatment. Third: plates whose substance shows a visible clouding — an effect which always appears in artificial solutions, indicating an incipient coagulation of the contractile tissue — show typically a more rapid rhythm than do the unclouded transparent plates, and the movement is more continuous and less easily inhibited. As described in my former paper, this effect appears very rapidly in pure isotonic solutions of alkali and alkali earth salts; in the above solutions it appears more gradually and is usually not evident until at least several hours have elapsed; but even in sea water it is found eventually and is accompanied by a similar alteration in the character of the movement. Fourth: in addition to the above specific effects of the calcium there is always apparent a well-marked antitoxic action, — the presence of calcium in favorable proportions prolongs the period during which the plates remain living and capable of normal activity; *i. e.*, the medium is rendered more favorable, in accordance with a rule of very general applicability.

To summarize: calcium ions evidently inhibit the contractile process in media similar to sea water in constitution, especially under conditions of mechanical disturbance; this inhibition becomes less effective after the plates have been immersed for some hours in the solution, and the diminution in effectiveness appears to run parallel with the progress of coagulative changes in the substance of the

plate. Now, coagulative changes appear in some manner to underlie contraction, as indicated by the association of an accelerated rhythm with visible changes of this kind in the contractile fibrillæ; hence the inference is suggested that the diminishing inhibitive effect is dependent on a progressive ascendancy of conditions favoring coagulative and so contractile processes in the fibrillæ. Calcium thus appears to exercise its action by influencing the velocity or the character of certain coagulative changes intimately associated with the act of contraction. Later I shall attempt to account theoretically for this connection between coagulative changes in the fibrillæ and contraction; the mechanism of inhibition will then be again considered and a definite theory proposed (see page 214 *seq.*).

It is remarkable that calcium cannot be replaced in this relation by either strontium or barium; its action has thus a certain specificity difficult to explain on physico-chemical grounds. Two series of solutions similar to the above were tried in which strontium and barium chlorides were respectively substituted for calcium chloride, *viz.*, 100 volumes van't Hoff's solution (without sulphate) + 0.5, 1, 2, 4, and 8 volumes  $m/2$   $\text{SrCl}_2$  or  $\text{BaCl}_2$ . In the first four solutions of the strontium series mechanical inhibition was almost entirely absent. In Solution 5 (+ 8 c.c.  $m/2$   $\text{SrCl}_2$ ) the plates, after an hour in the solution, showed active movement of a more intermittent character than in the four preceding; stirring and poking with the glass rod produced, however, only slight and doubtful inhibition; some hours later no sign of inhibition could be seen, and the only evident effect was one of stimulation. With barium chloride the plates in the first three solutions showed, after an hour, active movement not inhibited but stimulated by mechanical treatment; the movement lasted for more than twenty-four hours and always showed this form of response. In Solution 4 (+ 4 c.c.  $m/2$   $\text{BaCl}_2$ ) the toxic influence of the barium soon became evident; after an hour the plates were inactive and partly coagulated; later a few plates became active; contact then showed no inhibitory action, but caused well-marked stimulation. With 8 volumes  $m/2$   $\text{BaCl}_2$  no movement resulted, and coagulation was rapid.

*Potassium salts.*—The presence of potassium is inessential to this form of inhibition. A small proportion of potassium, as of calcium, is favorable to the preservation of irritability in the plates. Thus in one series (September 15, 1906) all the plates in the van't Hoff's solution (with  $\text{MgSO}_4$ ) were transparent and active after

twenty-three hours; in the corresponding solution without sulphate conditions were similar, and a few plates remained active for so long as fifty hours. In a mixture containing sodium and magnesium chlorides in about the same proportions (90 volumes  $m/2$  NaCl + 10 volumes  $m/2$   $MgCl_2$ ) movement had ceased and the plates were coagulated after nine hours. In the same solution with the addition of 1, 2, 3, and 4 volumes, respectively, of  $m/2$  KCl, the results were: in the first movement lasted more than eight hours; in the second some plates remained active after twenty-nine hours; the other two were less favorable, but movement was considerably prolonged in both.

The insusceptibility to mechanical inhibition in mixtures of sodium and magnesium chlorides is not altered by the addition of even large quantities of potassium chloride. This is illustrated by the following series. Rows of plates from *Mnemiopsis* were placed in these solutions: (1) 90 volumes  $m/2$  NaCl + 10 volumes  $m/2$   $MgCl_2$  (2-5) 100 volumes Solution 1 + respectively 1, 2, 4, and 8 volumes  $m/2$  KCl. In the first four solutions the plates after two hours and thirty minutes were vigorously active, and were stimulated but not perceptibly inhibited by agitation and contact; in the fifth, after the same interval, several plates had undergone coagulation, and the movement in those still living was enfeebled; here also contact stimulated activity with no sign of inhibition. The conditions in this contractile tissue seem thus to differ fundamentally from those in the vertebrate heart, where, according to the researches of Howell, potassium salts play an essential rôle in vagus inhibition.<sup>5</sup>

Addition of calcium to this mixture of sodium and magnesium chlorides has the same effect as in the preceding series with van't Hoff's solutions. The following series will illustrate:

Strips with attached plates (*Mnemiopsis*) were placed in the following solutions at 3.50 to 3.55 P. M., September 11, 1906. A mixture of 90 vols.  $m/2$  NaCl + 10 vols.  $m/2$   $MgCl_2$  was used, to which  $m/2$   $CaCl_2$  was added in the following proportions:

1. Control without Ca: After one hour movement is active; it is stimulated and not inhibited by contact. After three hours about one third of the plates are coagulated; the remainder show the same reaction as before.
2. 100 vols. solution + 0.5 vol.  $m/2$   $CaCl_2$ : After an hour movement is active; it is not inhibited but stimulated by contact. One or two plates are coagulated after three hours; next morning (eighteen hours) all are coagulated.

<sup>5</sup> HOWELL: This journal, 1906, xv, p. 280.

3. 100 vols. solution + 1 vol.  $m/2$   $\text{CaCl}_2$ : After one hour movement is active and shows only slight temporary inhibition. The plates are unaltered after three hours; next morning all are coagulated.
4. 100 vols. solution + 2 vols  $m/2$   $\text{CaCl}_2$ : After one hour movement is active but intermittent and is easily inhibited; otherwise like Solution 3.
5. 100 vols. solution + 4 vols.  $m/2$   $\text{CaCl}_2$ : After one hour the plates show little spontaneous movement; this is very readily inhibited.
6. + 8 vols.  $m/2$   $\text{CaCl}_2$ : After one hour the plates are inactive, with the exception of two at the end of a strip which are partly coagulated and show the active vibratory movements (not inhibited) typical of this condition.

A similar series with  $m/2$   $\text{SrCl}_2$  instead of  $\text{CaCl}_2$  showed active movement in the first four solutions (corresponding to Solutions 2 to 5 above); the plates in Solution 5 (with 4 volumes  $m/2$   $\text{SrCl}_2$ ) showed some slight inhibition after an hour in the solution, but for the most part no perceptible inhibition followed contact. In Solution 6 (+ 8 volumes  $m/2$   $\text{SrCl}_2$ ) the plates were inactive and partly coagulated after an hour. Here again, as in the preceding series, strontium proves incapable of replacing calcium, although when it is added in considerable concentration some slight inhibitory action is seen.

The effects of a progressive increase in the proportion of calcium are illustrated in more detail by the results of the following series:

A solution consisting of 90 vols.  $6/10$   $m$   $\text{NaCl}$  + 10 vols.  $m/2$   $\text{MgCl}_2$  was used, to which  $m/2$   $\text{CaCl}_2$  was added in the proportions indicated. Strips with rows of plates from *Mnemiopsis* were introduced into the solution at the times indicated, from 11.05 to 11.13 A. M., August 24, 1905.

1. 90 vols.  $6/10$   $m$   $\text{NaCl}$  + 10 vols.  $m/2$   $\text{MgCl}_2$ : Strips introduced 11.05 A. M. Movement begins very soon after immersion in the solution; at 11.14 it is active and almost perfectly continuous. At 11.23 contact with a glass rod or jarring the dish produces practically no effect; violent shaking may, however, produce a slight momentary inhibition; active movement is resumed almost immediately. At 11.50 the solution was changed for fresh to remove the calcium salts derived from the strips; at 11.52 the movement is continuously active and continues vigorously in spite of violent stirring, shaking, and jarring. At 12.23 the plates show incipient coagulation, and movement is enfeebled; there is no mechanical inhibition. Most of the plates are coagulated at 12.45.
2. 99 vols. Solution 1 + 1 vol.  $m/2$   $\text{CaCl}_2$ : Introduced 11.07. At 11.14 movement is active and somewhat intermittent, ceasing every now and then for a few seconds. At 11.25 to 11.30 a slight touch with the rod is

- followed by instant inhibition; movement is resumed almost immediately (within one to three seconds). Jarring the dish inhibits less readily than in Solution 3. At 12.00 and 12.24 movement is active and more or less intermittent; it is easily inhibited as before. At 2.00 a fair proportion of the plates are coagulated; the rest show active vibratory movement, which is inhibited less readily than before. At 3.20 all the plates are coagulated.
3. 98 vols. Solution 1 + 2 vols.  $m/2$   $\text{CaCl}_2$ : Introduced 11.08. At 11.15 movement is active and intermittent, of slower rhythm than in Solution 2, with pauses of considerable length. At 11.25 slight contact with the rod produces inhibition lasting several seconds; the plates are more sensitive than in Solution 2; jarring the dish readily inhibits all activity. At 12.00 movement remains active, though with a somewhat slower rhythm than in Solution 2; it is easily inhibited, and inhibition lasts longer than in Solution 2. At 12.25 and 12.45 conditions are similar. At 3.20 all plates are coagulated.
  4. 95 vols. Solution 1 + 5 vols.  $m/2$   $\text{CaCl}_2$ : Introduced 11.09. At 11.16 the plates show occasional beats; the rhythm is slow and irregular, with relatively prolonged periods of quiescence. At 12.00 the plates show fairly active intermittent movement more continuous than at first: slight contact very readily produces inhibition which lasts longer than in the preceding solution. At 12.25 and 12.45 active intermittent movement continues, easily inhibited as before. At 2.00 all the plates are coagulated.
  5. 90 vols. Solution 1 + 10 vols.  $m/2$   $\text{CaCl}_2$ : Introduced 11.10. The plates remain inactive; no movement is seen. At 12.00 the plates are already whitened and show no movement.
  6. 80 vols. Solution 1 + 20 vols.  $m/2$   $\text{CaCl}_2$ : Introduced 11.12. At 11.20 the plates are already whitened and show no movement.
  7. 70 vols. Solution 1 + 30 vols.  $m/2$   $\text{CaCl}_2$ . Similar to Solution 6.

Another series with the same solutions gave an identical result; the movement began immediately in Solution 1 and continued in rapid and continuous rhythm without inhibition; in Solution 2 movement also began at once, but was more intermittent; in Solution 3 it began after a short interval of quiescence and continued intermittently in waves of activity alternating with long pauses; in Solution 4 no movement appeared for the first three or four minutes; after five minutes an occasional beat was seen, but the plates remained inactive for the greater part of the time. Corresponding to the increase in the initial period of quiescence was the typical increase in the readiness of mechanical inhibition: in Solution 2 jarring the dish produced momentary inhibition lasting at most for two or three seconds; in Solution 3 the same treatment was followed by a pro-

longed pause, while in Solution 4 spontaneous movement, when it occurred, was still more readily inhibited, and the resulting period of inactivity was greatly prolonged.

A third similar series showed the same increasing suppression of spontaneous movement with increasing proportions of calcium, simultaneously with an increasing susceptibility to mechanical inhibition. In all of these series the addition of a favorable proportion of calcium prolonged markedly the period during which the plates remained living and capable of activity.

Two additional series of experiments were performed in which the calcium chloride in the above solutions was replaced by strontium chloride, with results similar to those already described. Addition of strontium in large proportions — up to 70 vols. Na-Mg solution + 30 vols.  $m/2$   $\text{SrCl}_2$  — was found not to prevent the early appearance of spontaneous movement in the unaltered plates; and while mechanical inhibition is not entirely absent in solutions containing ten or more volumes of the  $m/2$   $\text{SrCl}_2$  solution, it is relatively slight and temporary. In solutions with 95 vols. Na-Mg mixture + 5 vols.  $m/2$   $\text{SrCl}_2$  little or no mechanical inhibition was seen. The general conclusion, therefore, indicated by these and the above experiments, is that strontium, while capable to a slight degree of performing the inhibitory function of calcium, is incomparably less effective than the latter cation. The same appears to be true of barium. Systematic experiments with salts of other metals have not yet been tried.

*Conditions under which calcium exercises its inhibitory function.* — The above relation of calcium to inhibition is not absolute, but is found, so far as my observation has gone, only in solutions containing also sodium and magnesium salts in favorable proportions. Sodium salts cannot be replaced by those of other alkali metals, as potassium and ammonium. I have not yet examined the case of lithium, whose similarity to sodium (in relation to contractile processes) might lead one to expect the possibility of at least a partial substitution. The presence of magnesium in addition to sodium is essential; salts of this metal are by far the most favorable in counteracting the destructive action of pure solutions of sodium salts on this tissue; the only other metal that approaches magnesium in this respect is manganese. It is remarkable that the alkali earth metals, calcium, strontium, and barium, have only slight antitoxic action with pure solutions of sodium salts in the absence of magne-



sium. This is illustrated by the following experiments with the swimming-plates of *Eucharis multicornis*. Strips with plates attached were placed in the following solutions: (1) pure  $6/10\ m$  NaCl; (2) 90 vols.  $6/10\ m$  NaCl + 10 vols.  $m/2\ CaCl_2$ ; (3) 80 vols.  $6/10\ m$  NaCl + 20 vols.  $m/2\ CaCl_2$ , etc., in regular series to pure  $m/2\ CaCl_2$ . In the pure  $6/10\ m$  NaCl the typical effect already described is seen, — rapid vibration accompanied by coagulation of the plate substance. In Solution 2 (90 NaCl + 10  $CaCl_2$ ) active vibratory movements also begin at once; the movement is unaffected by mechanical disturbance, and is accompanied, though more gradually than in the pure NaCl solution, by progressive coagulation of the fibrillæ. In the other solutions of the series and in the pure  $CaCl_2$  solution typical rapid vibratory movements also occur accompanied by clouding and coagulation. The antitoxic action with calcium alone is thus slight, although, on the whole, movement is slower and more prolonged in mixtures of sodium and calcium chlorides than in the pure sodium chloride. Nevertheless in all cases activity ceases within half an hour or less, and susceptibility to mechanical inhibition is absent in spite of the large proportion of calcium in these solutions. The difference from magnesium is striking: addition of magnesium chloride to pure  $6/10\ m$  NaCl results, as already seen, in typical well-marked antitoxic action; the movement has the normal metachronous beating character and in favorable solutions may last several hours. Such movement, as already emphasized, is not susceptible to mechanical inhibition, but becomes so on the addition of a little calcium to the solution.

In sea water the maintenance of the normal rhythm thus depends on the presence of the magnesium, while the intermittence and ready inhibition of the movement depend on the calcium; the potassium, in some manner not yet clear, constitutes an important element in the general favorability of the medium. Increase of the proportion of calcium over the normal depresses spontaneous contractility and increases the readiness and persistency of the response to inhibitory influences; while decrease in calcium has the reverse effect, — accelerating the normal rhythm and rendering it more continuous, and removing to a greater or less degree the inhibitory response to mechanical stimuli.

The conditions of activity in such a tissue evidently depend intimately on the ionic constitution of the medium. Thus mechanical stimuli affect the contractile activity in the presence of calcium in



a precisely opposite manner from that shown in its absence — checking activity under the former conditions and stimulating it under the latter. In sea water contact usually checks activity in healthy plates; but an intermediate condition in which contact sometimes accelerates and sometimes checks activity is often seen in sea water, especially in plates that have been kept in this medium for some time. A fundamental similarity in the nature of the two apparently opposite processes of stimulation and inhibition is indicated; whether a given stimulus increases or depresses activity depends on the nature and proportions of the ions in the tissue.

The repressive influence of the calcium ion on spontaneous rhythmical activity in muscle has been emphasized especially by Loeb;<sup>6</sup> the effect which he describes is similar in some respects to that seen in the swimming-plate in solutions of sodium and magnesium chlorides. The conversion of a stimulating into an inhibitory action through an increase in the concentration of the calcium ions in the tissue is, however, in some respects a quite distinct phenomenon. There is something analogous also in the behavior of the veratrinized vertebrate muscle, where stimulation accelerates the relaxation, i. e., inhibits an existing state of contraction. The case of the vertebrate heart shows the closest analogy. Howell<sup>7</sup> has found that vagus inhibition is dependent on the presence of potassium; calcium is comparatively inessential, although it appears, at least under certain conditions, to play some part. Increasing the proportion of potassium ions in the perfusing solution increases the sensitivity to vagus inhibition, while their removal lessens or abolishes this effect. Here also a dependence of inhibition on the presence of a specific ion is indicated; in both this case and the above the active cation is one whose presence in the medium in larger than the normal proportions depresses the contractile activity. A possible inference, and one suggested by Howell, is that the stimulus in some way sets free the depressant ion within the tissue.

Just why, in the Ctenophore swimming-plate under normal conditions, this specific depressant influence of calcium should especially manifest itself under mechanical stimulation, but usually not at other times, requires explanation. If the depression of contractile activity is due to an increase in the proportion of calcium ions in the contractile tissue, one can only assume that the mechanical stimulus acts

<sup>6</sup> LOEB: *Festschrift für Fick*, 1899, Braunschweig, p. 101.

<sup>7</sup> HOWELL: *Loc. cit.*

by facilitating the entrance of these ions, presumably by altering the permeability of the surface layer and thereby rendering the entire structure more readily penetrable. If we assume this, we must also suppose that the active plate offers normally a certain resistance to the entrance of calcium ions, since otherwise the continued beating in sea water would be inexplicable. But why should the mechanically induced inhibition be only temporary, and the less so the higher the proportion of calcium in the medium? Only a hypothetical explanation can be offered at present; it is probable that under normal conditions a state of equilibrium exists within the plate involving a certain relatively fixed proportionality in the calcium content of the two structural elements of the tissue, — the contractile fibrils and the interfibrillar substance. We may assume that when the tissue is placed in a medium with more than the normal concentration of calcium salts calcium ions enter the interfibrillar spaces; if the tissue is agitated in such a medium, or even one of normal calcium content, the same may be supposed to occur. The presence of the resulting excess of calcium ions in the interfibrillar spaces inhibits activity until the normal state of equilibrium is restored, a process requiring some time. Increasing the calcium content of the medium naturally increases the time required for the attainment of this equilibrium, and hence inhibition is more prolonged. Above a certain limiting concentration of calcium the normal equilibrium cannot be attained and the inhibition is permanent. Whether such a conception corresponds to the actual conditions or not, it seems necessary to assume some alteration in the distribution of the inhibiting ions within the tissue in order to explain the evanescence of the effect; and it is only to be expected that the time required for such redistribution will be the more prolonged the greater the number of inhibiting ions entering the tissue.

The part played by the calcium in inhibition requires further analysis, and it is clear that the problem involves the further fundamental question of the nature of the contractile process itself. The above facts appear to throw some light on both questions; they indicate that the essential action of the calcium lies in its altering the permeability of the contractile elements to ions. Thus it seems certain that ionic interchanges between contractile tissues and the medium constitute an important condition of activity in such tissues; this seems clearly demonstrated by the existence of an action current; during contraction the polarization at the surface of the contractile elements

diminishes or disappears, and ions pass freely in both directions, as shown by the passage of the current. The seat of the electromotive force in muscle is usually considered to be the surface layer or plasma membrane of the entire cell; but a large portion of the potential probably has its origin at the boundary layers between the numerous contractile elements or fibrillæ and the sarcoplasm,<sup>8</sup> since each contractile element must be regarded as having the same electrical properties as the entire compound structure. There is no reason to doubt that the facts established for muscle apply also to a relatively simple contractile element like a cilium, in which there is a similar fundamental structure, namely, parallel contractile fibrils imbedded in an interstitial substance analogous to the sarcoplasm of a muscle fibre. An action current must then be supposed to accompany each ciliary beat as in the case of each heart beat, — that is, there is a temporary depolarization, with passage of ions in both directions through the boundary surfaces.

From this point of view the above cessation of movement in calcium-containing media evidently indicates a cessation of such ionic interchanges. The influence of the calcium ions on the permeability of the surface layers of the contractile fibrillæ is thus indicated as an important condition of inhibition. Evidently increasing the concentration of calcium ions retards such ionic interchanges, — as shown by its slowing the rhythm and under certain conditions, as mechanical agitation, checking it altogether. In other words, the calcium acts by decreasing the permeability of the surface layers to ions. On the other hand, the fact that when the plate begins to undergo *ante-mortem* coagulation, its rhythm becomes faster and its susceptibility to inhibition decreases, indicates an *increased* readiness of such ionic interchanges under these conditions. It is significant that this change in activity is associated with a visible change in the condition of the plate, namely, the coagulative change already described; such a change, by impairing the continuity of the surface layers at which the ionic interchanges occur, must greatly accelerate such interchanges, and as a natural consequence the dependent rhythm of contraction is also accelerated. These facts afford very direct indication that the rate of the contractile rhythm depends on the degree of permeability of the contractile elements to ions.

The connection between coagulative changes in the fibrils and con-

<sup>8</sup> Cf. BERNSTEIN: *Archiv für die gesammte Physiologie*, 1902, xcii, p. 521, and "Die Kräfte der Bewegung in der lebenden Substanz," Braunschweig, 1902.

traction is, however, undoubtedly farther reaching than this, and involves alterations in the entire substance of the fibrillæ and not merely in their surface layers. During the abnormally accelerated vibratory movement in pure 6/10 NaCl or similar media the *whole* fibril coagulates, at a rate which is clearly proportional to the energy of the vibratory movement.<sup>9</sup> What are the conditions of this coagulative change, and why is it correlated with the contraction? Since coagulation of colloids is due to ions, we are led to consider the possibility that the *change in the ionic content of the fibril due to the ionic interchange* just discussed is the direct cause of the coagulative change producing contraction. This coagulative change seems independent of the entrance of specific ions from outside, since it occurs whenever the above abnormally rapid vibratory movement is established, whatever the character of the solution; *even in pure non-electrolyte solutions* (dextrose, cane sugar, urea) *the same effect is seen*.<sup>10</sup> The coagulation must then be referred to alterations in the concentration or nature of the ions or ion-producing substances already present in the tissue, and we are led to consider, first, the relations of equilibrium between these ions and the colloids constituting the contractile fibril, and, second, the possible consequences of an alteration of this equilibrium incident on the above change in the ionic permeability of the fibril.

In the inactive fibril an equilibrium must exist between its colloids on the one side and its contained ions on the other. While this equilibrium persists unchanged, the colloids preserve unchanged a certain aggregation state, and no contraction will occur. In any system containing a hydrophilous colloid,<sup>11</sup> such as these composing tissues, a certain fairly well-defined state of colloidal aggregation corresponds to a given electrolyte content. This is best shown by the fact that the osmotic pressure of such solutions — which is an index of the number of independent active colloid particles in the solution — varies in a definite manner with variations in the electrolyte content.<sup>12</sup>

<sup>9</sup> Cf. my former paper (*loc. cit.*) pp. 125, 126.

<sup>10</sup> The plates of Eucharis may show active vibration in non-electrolyte solutions, although the movement is less energetic than in salt solutions. Slight acidulation of the solution produces a marked increase in the vigor and activity of such movements, as also in the case of salt solutions. The presence of ions in the external medium seems thus less essential for ciliary than for muscular contraction.

<sup>11</sup> HOEBER: *Physikalische Chemie der Zelle und der Gewebe*, 2te Aufl., Engelmann, Leipzig, 1906, p. 239.

<sup>12</sup> R. LILLIE: This journal, 1907, xx, p. 127.

Expressed somewhat differently, we may say that the nature and extent of the interface between colloid-rich and colloid-poor phases (or colloid particles and medium) is a function of the ionic content of the system; to a particular concentration of a given electrolyte a particular condition of equilibrium corresponds. We may therefore suppose that in the individual contractile fibril (considered as such a system) this equilibrium is determined by the character and surface extent of the colloidal constituents on the one hand, and the nature and concentration of the ions on the other. This entire system will be in equilibrium with the surrounding medium, — so far as it is not isolated from the latter by its surface layer, which forms a partition impermeable to all of its colloids, and to a large proportion of its contained ions. If now the partition suddenly becomes permeable to ions, an ionic interchange between medium and fibril at once occurs. The electrolyte content of the fibril is thus altered, and the previous equilibrium is disturbed; a change in the aggregation state of the colloids at once follows. This last event, according to the hypothesis, is the direct condition of the contraction.

The importance of the difference between the respective ionic contents of contractile tissue and medium is thus evident: it supplies the conditions for the polarization of the surface layers, as in a "dialytic" cell,<sup>13</sup> and also favors the ionic interchange as soon as the partition between medium and tissue becomes permeable. At this moment the ionic interchange occurs; the movement of ions is more rapid than that due to mere diffusion, on account of the potential gradient already existing between exterior and interior of the fibril, and a correspondingly prompt aggregation change in the fibrillar colloids will follow. This aggregation change with the associated contraction is thus merely a response to a sudden alteration of the conditions of equilibrium. It is known that a *gradual* alteration of the ionic concentration is relatively ineffective as a stimulus, as also in changing the aggregation state of colloids.<sup>14</sup>

The relaxation remains to be accounted for; in most tissues it follows automatically with cessation of the stimulus. In the first place, it must be noted that the cessation of the action current indicates a restoration of impermeability, and relaxation must in some

<sup>13</sup> Cf. BRÜNNING: Archiv für die gesammte Physiologie, 1903, c, p. 367.

<sup>14</sup> Cf. FREUNDLICH: Zeitschrift für physikalische Chemie, 1903, lxiv, p. 129; HOEBER and GORDON: Beiträge zur chemischen Physiologie und Pathologie, 1904, v, p. 432.

way depend on this. Apparently relaxation indicates a return to the normal resting condition of equilibrium as soon as the possibility of ionic interchange with the medium is removed. In addition to this relatively passive side of the phenomenon there may be a more active side; no doubt the completeness of the reversibility of the contractile change is in some way connected with the metabolic processes in the tissue.

These metabolic processes are of course the ultimate source of the energy of contraction, which thus represents chemical energy transformed into the surface energy of the colloidal fibrils.<sup>15</sup> In a tissue contracting rhythmically and automatically, like the heart muscle or the cilium, we may infer that the periodic alteration in the permeability of the contractile elements is due to the production of certain ions through metabolic, preponderantly oxidative, processes. These ions leave the tissue not continuously, but rhythmically, owing to their own action on the permeability of the surface layer, which automatically undergoes alternations of greater and less permeability, corresponding respectively to the contraction and the relaxation phases of the beat. Presumably a certain critical concentration of ions is necessary to reduce the impermeability sufficiently to allow the passage of the ions concerned and so to cause contraction. The contraction itself or the accompanying transfer of ions apparently involves a restoration of the original condition of rest, and so the cycle is repeated in regular rhythm. Since the alternation of permeability and impermeability corresponds to a successive polarization and depolarization of the boundary surfaces, the long-known electrical rhythm accompanies the rhythm of contraction.

As to the general mechanism of inhibition — the special phenomenon considered in this paper — its determining condition on the present theory is evident. In any rhythmically contracting tissue, like the cilium or the heart, the possibility and the rate of the ionic interchanges on which contraction depends are dependent on the ionic permeability of the surface layer of the contractile fibril; this may be altered by ions in the external medium. The influence of calcium on the rhythm of the Ctenophore swimming-plate is an instance of this nature, as already explained, the calcium ion diminishing or abolishing the permeability.

<sup>15</sup> D'ARSONVAL, BERNSTEIN, and J. LOEB have supported similar views. Recently FREUNDLICH, "Kapillarchemie und Physiologie," Dresden, 1907, has given a striking exposition of this general theory.



The general physiological significance of these changes in permeability during activity seems to have been insufficiently emphasized. In all probability they constitute an important factor in the intake of dissolved substances and especially food materials by living tissues. Thus the difficulty (on which Overton<sup>16</sup> has insisted) in accounting for the entrance of such substances as sugars — for which the plasma membrane of resting cells is impermeable — is overcome. Instead of supposing that these substances form lipoid-soluble combinations with other compounds, we have only to assume that they enter the cell during its active condition, that is, at a time when the plasma membrane is demonstrably freely permeable to ions, and supposedly also for other substances that are unable to enter the resting cell.<sup>17</sup> The functional hypertrophy of active muscle thus becomes intelligible: with the increased blood supply accompanying activity and due to vascular dilatation there is associated an increased permeability of the plasma membranes; food materials are thus enabled readily to enter the cell. Hence, also, the progressive atrophy accompanying long-continued inactivity; such a muscle actually starves in the midst of plenty simply on account of the impermeability of its surface layers to the necessary food materials.

#### SUMMARY.

1. Mechanical stimulation arrests the automatic activity of the Ctenophore swimming-plate in sea water and in certain artificial media similar to sea water in general composition (van't Hoff's solution, mixtures of NaCl and  $MgCl_2$ ).

2. This susceptibility to mechanical inhibition is dependent on the presence of calcium salts; decrease in the proportion of calcium increases the automatic activity of the plates and diminishes and finally altogether removes the above susceptibility; increase in calcium diminishes automatic activity and greatly facilitates inhibition. Calcium cannot be replaced by strontium or barium.

3. The essential action of the calcium in producing this effect appears to consist in an alteration of the permeability of the contractile tissue to ions. The contractile activity is explained as due to

<sup>16</sup> OVERTON: *Archiv für die gesammte Physiologie*, 1902, xcii, p. 215.

<sup>17</sup> This suggestion has been made by HOEBER: *Archiv für die gesammte Physiologie*, 1905, cvi, p. 633, and *Physikalische Chemie der Zelle und der Gewebe*, 2te Aufl., 1906, p. 318.



rhythmical alterations in the ionic content of the contractile fibrils, due to rhythmical alterations in their ionic permeability, — of which the action current is evidence. Aggregation changes in the colloidal constituents of the fibrils accompany these rhythmical changes in their ionic content and form the direct condition of the rhythmical contraction. Calcium salts check or inhibit contractile activity by decreasing the ionic permeability of the fibrils; mechanical stimulation acts by facilitating the entrance of calcium ions into the tissue.

## THE INFLUENCE OF VARIOUS LYMPHAGOGUES ON THE RELATIVE CONCENTRATION OF BACTERIO- AGGLUTININS IN SERUM AND LYMPH.

BY B. BRAUDE AND A. J. CARLSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

IT is well known that the natural as well as the specific bactericidal and bacterio-agglutinating substances of the blood are found in the lymph of the same animal. In the experiments summarized in this report we endeavored to determine (1) the relative concentration of the bacterio-agglutinin in dog's serum and in dog's lymph from different regions of the body; and (2) the change in this relation of serum and lymph produced by various lymphagogues. The work is an extension of the investigation of the influence of lymphagogues on the hemolytic action of the lymph by Hughes and Carlson. The problem is stated in greater detail, and its complexity discussed, in the paper by Hughes and Carlson, to which the reader is referred.<sup>1</sup>

### I. THE LITERATURE.

The earliest work on the bactericidal action of the lymph and other body fluids was done by Nuttall,<sup>2</sup> Prudden,<sup>3</sup> and Meltzer and Norris.<sup>4</sup> Meltzer and Norris studied the bactericidal action on the typhoid bacillus of the lymph from the thoracic duct of the dog, making use of the plating method. The lymph was obtained from fasting dogs and was therefore free from fat. These investigators conclude that dog's thoracic lymph has the same or nearly the same

<sup>1</sup> HUGHES and CARLSON: This journal, 1908, xxi, p. 236.

<sup>2</sup> NUTTALL: Zeitschrift für Hygiene, 1888, iv, p. 353.

<sup>3</sup> PRUDDEN: Medical record, 1890.

<sup>4</sup> MELTZER and NORRIS: Journal of experimental medicine, 1897, ii, p. 701.

bacteriocidal power as dog's serum. Their observations did not extend to the agglutinins.

Pick<sup>5</sup> and Levy and Giessler<sup>5</sup> have described bacterio-agglutinins in the cerebrospinal fluid, aqueous humor, pericardial fluid, and various transudates and exudates in man. Other observers have failed to find agglutinins for bacillus typhosus in the cerebrospinal fluid of typhoid patients. These agglutinins are probably, therefore, not present in all cases. Kohler,<sup>6</sup> for example, secured positive results in three out of ten typhoid patients, while in the cerebrospinal fluid of ten non-typhoid patients positive results were obtained in only one case. The same observer records the presence of agglutinins in the blood of non-typhoid patients as well as healthy individuals to the extent of 15 per cent of the subjects investigated. So far as we know, there is no evidence that this agglutinin is specific for the typhoid bacillus. Kohler observed an increase in the natural agglutinating power of the blood of rabbits and dogs after obstruction of ductus choledoches, due in all probability to the accumulation of bile acids (taurocholic acid) in the blood. By intravenous injection of taurocholic acids the concentration of normal typhoid agglutinins is greatly increased in the dog's blood, but not in that of the rabbit.

Rosenberg<sup>7</sup> studied the concentration of the typhoid agglutinins in the body fluids of the rabbit immunized against the typhoid bacillus. The concentration of the agglutinins in the various transudates depends on their concentration in the blood. On obstructing the ureters the concentration of the agglutinins in the transudates is increased. Acites fluid, pleural and pericardial fluids, contain a greater percentage of the agglutinins than aqueous humor or cerebrospinal fluid.

According to zur Nedden,<sup>8</sup> typhoid bacteriolysins of the blood do not pass into the lachrymal fluid, nor are they present in the normal secretion of the conjunctiva, but the catarrhal secretion of the conjunctiva is strongly bacteriocidal, and this action is due, at least in part, to lysins in solution.

<sup>5</sup> Cited from the extensive paper on the agglutinins by KOHLER, *Klinisches Jahrbuch*, 1902, viii, p. 84.

<sup>6</sup> KOHLER: *Loc. cit.*

<sup>7</sup> ROSENBERG: cited from *Centralblatt für Bakteriologie*, 1905, xxxvi, p. 193.

<sup>8</sup> ZUR NEDDEN: *Zeitschrift für Angeheilkunde*, 1907, xviii, p. 300.

## II. METHODS.

The lymphs were collected through sterile cannulae, with the animals under light ether narcosis. The lymphs were allowed to coagulate in the sterile test tubes used for their reception, and the tests were made on the fluid expressed from the light coagulum. The blood was likewise collected through sterile cannulae, invariably centrifugalized, and the tests made on the fluid expressed from the corpuscle-free part of the clot. The cerebrospinal fluid, aqueous humor, and pericardial fluid were usually collected after bleeding the animals to death, so as to render admixture with blood more remote. When erythrocytes were present in either of these fluids, the fluid sample was discarded.

The lymphs and sera were usually drawn from animals after having been starved for from twenty-four to thirty-six hours in order to obviate the presence of a great amount of fat in emulsion.

Most of the tests were made on the typhoid agglutinins present in the blood and lymph of normal dogs. Tests were also made on the body fluids of four dogs and two cats after previous inoculation with the typhoid bacillus. The dogs were inoculated intraperitoneally three times, fourteen, eight, and three days prior to the experiment, with twenty-four hours old live broth culture of the bacillus, in quantities of 5 c.c., 7 c.c., and 10 c.c. respectively. The cats were similarly immunized on the tenth and fourth day preceding the experiments with 4 c.c. and 7 c.c. of the culture.

Most of the tests were made on the same day the body fluids were secured. In the cases where the lymphs and sera could not be tested immediately after being drawn, the material was kept in the ice box. Sera and lymphs kept in this way in the ice box for as long as six days neither gained nor lost in their agglutinating power.

The hanging-drop method was used in all our experiments. The bacteria adhering to the point of a platinum needle were mixed with two loops full of sterile broth and one loop full of the serum or lymph in normal concentration or in varying degrees of dilution. The broth was introduced in order to have an abundant food supply for the bacteria. For each series of tests one control preparation was made using only sterile broth.

## III. RESULTS.

The serum, thoracic lymph, neck lymph, and pericardial fluid of normal dogs contain an agglutinin or agglutinins for typhoid bacilli. The agglutinin is most concentrated in the serum. Next in order comes the lymph from the thoracic duct. The neck lymph has a still weaker action, while the pericardial fluid has the least of any of the active liquids. The cerebrospinal fluid and the aqueous humor of normal dogs have no agglutinating action on typhoid bacilli. The relative agglutinating power of serum, thoracic lymph, and neck lymph of the normal dog thus exhibits the same relations as their hemolytic power, but the difference in their agglutinating action is not always as marked as that of their hemolytic power. When the body fluids are added to the broth-bacillus mixture in the proportion 1 : 2, all the bacilli are agglutinated by the serum in from ten to fifteen hours, while some bacilli remain motile in the thoracic and neck lymph for more than twenty-four hours.

Out of a total of thirteen normal cats examined, in only one case did the serum and lymphs show any agglutinating action on the typhoid bacilli. The sera and lymphs were added in the proportion 1 : 2, as in the case of the dog's body fluids, but there appeared no agglutination during the first twenty-four hours, at which time observations were discontinued. It is therefore probable that the body fluids of normal cats contain no agglutinins for the typhoid bacilli. This is rendered more likely because of the fact that the serum and lymphs of the cat giving positive results had as great agglutinating power as the body fluid of the two cats which were immunized against the bacilli. The one cat forming the exception had in all probability been infected with typhoid bacilli before he came into our hands. The cats were brought to the laboratory from all parts of the city, so we had no means of knowing their history.

The serum and lymphs of the cats immunized against the typhoid bacillus exhibited the same relation in agglutinin concentration as the corresponding fluids of the normal dogs, as will be seen from Series I, B.

The lymphagogues (strawberry extract, 10 per cent peptone, 10 per cent cane sugar, 5 per cent sodium chloride) have no effect on the relative concentration of the agglutinins in the serum and the lymphs (Series II). Our experiments on this point comprise three with the

peptone solution, one with strawberry extract, four with hypertonic sodium chloride solution, and two with hypertonic cane sugar solution. In no instance were we able to detect any diminution or in-

SERIES I.

The relative agglutinating power of serum and lymph from the thoracic duct and from the neck lymphatics.

Animal.	Time.	Serum.	Thoracic lymph.	Neck lymph.	Control.
A. Dog, not immunized.	5 min.	Slight agglutination, most bacteria motile.	Slight agglutination.	Slight agglutination.	Normal.
	60 min.	More extensive agglutination.	More extensive agglutination.	More extensive agglutination.	Normal.
	5 hrs.	Nearly complete agglutination, a few bacteria still motile.	Less agglutination than in serum.	Less agglutination than in thoracic lymph.	Normal.
	24 hrs.	Complete agglutination.	Most of the bacilli agglutinated.	Less agglutination than in thoracic lymph.	Normal.
B. Cat, immunized against the typhoid bacilli.	5 min.	Extensive agglutination.	Extensive agglutination.	Extensive agglutination.	Normal.
	60 min.	Nearly complete agglutination.	Less than in serum.	Less than in thoracic lymph.	Normal.
	5 hrs.	Complete agglutination.	Nearly complete agglutination.	Less than in thoracic lymph.	Normal.
	24 hrs.	Granulation and solution.	Complete agglutination.	Nearly complete agglutination.	Normal.

crease in the agglutinating action of the lymphagogue lymphs over that of the normal lymphs. On the mechanical theory of the action of these lymphagogues there ought to have been an increase in the agglutinating power of the peptone and the strawberry extract lymph, and a diminution in the sugar and the sodium chloride lymphs, inasmuch as the normal serum contains a slightly greater percentage of the agglutinins than the lymph. According to our results the rate of formation or appearance of the agglutinins in the lymph is in some way directly correlated with the rate of formation of the lymph itself.

The intravenous injection of these lymphagogues does not seem

to alter the agglutinating power of the serum itself. The test sample of lymphagogue serum was always taken about sixty minutes after the lymphagogue injection. The crystalloid lymphagogues

# SERIES II.

The relative agglutinating action of serum and lymph before and after intravenous injection of peptone. Time between the injection of the peptone and taking the test samples, 60 minutes.

Time.	Serum.		Lymph from thoracic duct.		Lymph from neck lymphatics	
	Normal.	After peptone injection.	Normal.	After peptone injection.	Normal.	After peptone injection.
A. DOG, NORMAL.						
5 min.	Partial agglutination, small clumps.	Same as in normal serum.	Slight agglutination.	Same as in normal.	Very slight agglutination.	Same as in normal.
60 min.	Greater agglutination, large clumps.	Same as in normal serum.	Less than in serum.	Same as in normal.	Less than in thoracic lymph.	Same as in normal.
5 hrs.	Almost complete agglutination.	Same as in normal.	Extensive aggl., but many bacilli still motile.	Same as in normal.	Less than in thoracic lymph.	Same as in normal.
24 hrs.	Complete agglutination.	Same as in normal.	Nearly complete agglutination.	Same as in normal.	Less than in thoracic lymph.	Same as in normal.
B. DOG, IMMUNIZED AGAINST THE TYPHOID BACILLUS.						
5 min.	Complete agglutination.	Complete agglutination.	Complete agglutination.	Complete agglutination.	Complete agglutination.	Complete agglutination.

increase the total amount of the blood by the passage of water from the tissues. This would lead to dilution of the agglutinins in the serum, unless agglutinins are also given up to the serum by the tissues. We may also have an increase in the rate of formation of agglutinins in the blood itself or a diminution in the rate of their destruction.



Hewlett<sup>9</sup> found that large doses of peptone introduced into the blood of a dog diminished its bactericidal action on the colon bacillus, weaker doses having no effect. We failed to observe any such action of peptone on the power to agglutinate the typhoid bacillus. But it must be remembered that the amount of the peptone injected in our experiments never reached 0.5 per kilogram body weight.

In normal dogs the pericardial fluid contains typhoid agglutinins, but in less concentration than the thoracic or the neck lymphs. The cerebrospinal fluid and the aqueous humor have no typhoid agglutinating action (Series III). In dogs immunized against the typhoid bacilli the agglutinins are present in considerable concentration in both the cerebrospinal fluid and the aqueous humor. There is considerable evidence to the effect that the normal cerebrospinal fluid is, at least in part, a product of secretion by the epithelial cells of the choroid plexus. The absence of the agglutinins in the normal cerebrospinal fluid and its presence after immunization may be due to special activity of the secretory cells under influence of the typhoid toxin; but it is also possible that the agglutinins, in virtue of their great concentration in the blood of the immunized dogs, simply are carried with the water current through the secreting cells into the cerebrospinal fluid. Their absence in normal animals, although present in the blood and the lymphs, and their presence in the immunized animals give us therefore no clew as to the place of formation of the agglutinins. The number and character of the leucocytes in the cerebrospinal fluid of the normal and the immunized dogs might aid in settling that question.

We have no evidence that the typhoid agglutinin in the serum and the lymph of normal dogs is specific. In fact, it is probably not specific. If the agglutinin is specific, all the dogs worked on must have had typhoid infection previous to being brought to the laboratory. This is, of course, possible, in the case of stray dogs in a large city, but it is hardly probable. If the agglutinin produced by immunization is new to the dog's organism, the presence of this substance in body fluids where the normal agglutinins do not occur may be related to differences in the character of the agglutinins themselves.

And yet there remains the suggestive fact that in cats, having no normal typhoid agglutinins, the immunization produces a much

<sup>9</sup> HEWLETT: *Archiv für experimentelle Pathologie und Pharmakologie*, 1903, xlix, p. 314.

## SERIES III.

The relative agglutinating action of serum lymph and the body fluids of normal and of immunized dogs.

Time.	Serum.		Lymph from thoracic duct.		Lymph from neck lymphatics.		Pericardial fluid.		Cerebrospinal fluid.		Aqueous humor.	
	Normal.	Immunized.	Normal.	Immunized.	Normal.	Immunized.	Normal.	Immunized.	Normal.	Immunized.	Normal.	Immunized.
5 min.	Partial agglutination.	Complete agglutination.	Partial agglutination.	Complete agglutination.	Partial agglutination.	Complete agglutination.	No agglutination.	Extensive agglutination.	No agglutination.	Extensive agglutination.	No agglutination.	Extensive agglutination.
60 min.	Extensive agglutination.	Complete agglutination.	Less than normal serum.	Complete agglutination.	Less than thoracic lymph.	Complete agglutination.	Slight agglutination.	Nearly complete agglutination.	No agglutination.	Greater agglutination.	No agglutination.	Greater agglutination.
5 hrs.	Nearly complete agglutination.	Complete agglutination.	Less than normal serum.	Complete agglutination.	Less than thoracic lymph.	Complete agglutination.	More extensive agglutination.	Complete agglutination.	No agglutination.	Greater agglutination.	No agglutination.	Greater agglutination.
24 hrs.	Complete agglutination.	Complete agglutination.	Nearly complete agglutination.	Complete agglutination.	Less than thoracic lymph.	Complete agglutination.	Extensive agglutination, but many bacilli still motile.	Complete agglutination.	No agglutination.	Nearly complete agglutination.	No agglutination.	Nearly complete agglutination.

weaker concentration of the agglutinin in the serum and the lymph than in the case of the dog. The serum and lymph of the immunized cats have about the same agglutinating power as the serum and lymph of normal dogs. The absence of agglutinating action of the body fluids of the normal cat and this relative weak concentration after immunization do not appear to be due to the presence of anti-agglutinins, because adding serum or lymph from normal cats to the corresponding fluids from dogs does not inhibit the agglutinating power of the latter.

THE RELATIVE RESISTANCE OF THE HEART GANGLIA, THE INTRINSIC NERVE PLEXUS, AND THE HEART MUSCLE TO THE ACTION OF DRUGS.

By W. J. MEEK.

[From the Hull Physiological Laboratory of the University of Chicago, and the Marine Biological Laboratory, Woods Hole.]

THIS report is a continuation of the work of Carlson on the point of action of drugs in the heart,<sup>1</sup> and was undertaken at Professor Carlson's suggestion. The particular point of this series of experiments is the relative rapidity by which drugs of the same concentration produce paralysis in the different tissues of the heart. It has been shown by Carlson that in *Limulus* the heart ganglion is much more sensitive to the action of drugs than is the heart muscle. In very weak concentrations the drugs act, therefore, primarily on the heart ganglion alone. The primary action of a drug on the ganglion may be the opposite from that on the muscle. The primary action of most drugs on the entire heart is the same in *Limulus* and in the vertebrates, but in the case of the vertebrate heart we are not able experimentally to confine the action of any drug to the ganglion alone, or to the heart muscle or the nerve plexus alone.

Such an attempt has been made by Rohde in the case of chloral hydrate.<sup>2</sup> Rohde found that in certain stages in the chloral hydrate action on the frog's heart the properties of the heart tissue appeared to be fundamentally altered. This was interpreted by Rohde as due to the complete paralysis of the intrinsic nervous tissue in the heart by chloral hydrate while the heart muscle still retained its excitability and contractility. Schultz and Carlson<sup>3</sup> have not been able to confirm Rohde on the point that the refractory state disappears com-

<sup>1</sup> CARLSON: This journal, 1906, xvii, pp. 1, 177.

<sup>2</sup> ROHDE: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liv, p. 104.

<sup>3</sup> SCHULTZ: This journal, 1906, xvi, p. 483; CARLSON: This journal, 1907, xviii, p. 71.

pletely in chloral hydrate narcosis before the disappearance of contractility. But Rohde's assumption that chloral hydrate completely paralyzes the heart nervous tissues before the heart muscle is substantiated by Carlson's observation on the *Limulus* heart. In that heart it can be proved directly that chloral hydrate in any given concentration paralyzes the heart tissues in the following order: ganglion, nerve plexus, muscle. According to my results this order holds for all drugs and chemicals tested; so in this regard chloral hydrate occupies no special position.

#### I. METHODS.

The various methods of preparing the *Limulus* heart for determining the action of chemicals on the different tissues have been described by Carlson and need not therefore be restated here. In many of the present experiments no special preparation of the heart was resorted to. The whole heart was immersed in the solution to be tested. The cessation of the rhythm was taken as indicating the point when complete paralysis of the ganglion had taken place. The lateral nerves or the nerve plexus as a whole was then tested by electrical stimuli from time to time, and the contractions observed in the anterior or posterior regions of the heart. Isolation of the lateral nerves is not necessary for this purpose, as the *Limulus* myocardium does not conduct the contraction under normal conditions. In most cases, however, the lateral nerves were isolated before being stimulated, so as to avoid even the possibility of muscular conduction or escape of the current to the heart muscle of the region used as the indicator. When the heart muscle no longer responded to the stimulation of the lateral nerves, the nerves were considered paralyzed. The stimulating electrodes were then shifted directly on to the muscle, and the time of complete disappearance of contractility determined. The point of complete disappearance of automatism and contractility was determined by aid of a hand lens, as it was found to be just as accurate as the graphic method, and had the advantage of being much simpler and more time-saving.

The drugs and chemicals used were of Merck's manufacture. The drugs were dissolved in sea water or serum. Sea water is isotonic and practically neutral to the heart. The salts were used in 6/10 *n* concentrations and diluted with sea water or serum.

The resistance of the heart's tissues to drugs and chemicals depends in part on the condition of the animal and the heart. The aim was to use hearts from vigorous and healthy animals only, so as to make the results in the case of the different drugs comparable.

## II. RESULTS.

In Table I are summarized the results on the drugs and chemicals so far tested. An examination of this table shows that the rate of paralysis of the three heart tissues is: ganglion, nerve plexus, heart muscle. There is no exception to this rule in the case of any chemical tested, or in any concentration of these chemicals. But in the case of some of the more intensely acting drugs or salts the greater the concentration, the more rapid the onset of paralysis, so that the difference in the element of time between the paralysis of the different tissues is greatly decreased, and hence not so readily determined.

The action of the drug as measured by the latent period in the onset of paralysis appears to be, on the whole, directly proportional to its concentration. For example, aconitin in concentration of 1/1000 stops the ganglionic automatism in about one minute, while the concentration of 1/2600 requires about three minutes to produce paralysis of the ganglion.

All of these drugs and chemicals act on all three of the heart tissues. The selective action is only one of degree. This principle is now gradually gaining recognition in vertebrate pharmacology and physiology, but much of the older work on atropin, nicotin, pilocarpin, curare, and other drugs fails to take into account the relative nature of the selective action.

The present results throw no light on the cause or causes of these differences in the three heart tissues to the deleterious action of drugs. It may be due to difference in permeability. The heart muscle is paralyzed quicker, for example, if the solutions are introduced into the heart cavity than if the collapsed heart is simply immersed in the solutions. When the latter method is employed, the chemicals must in part pass through the connective tissue sheath covering the surface of the heart before reaching the heart muscle. But even when introduced into the heart cavity, the drugs paralyze the nervous tissue before the muscle, so that, if it is a question of

TABLE I

Latent time in effecting paralysis of the *Limulus* heart tissues by certain drugs and chemicals. The figures represent the averages of the results on each drug.

Drug or chemical.	No. of exp.	Time for effecting paralysis, expressed in minutes.			Concentration of drug or chemical.
		Ganglion. (Automatism.)	Nerve plexus. (Excitability and conductivity.)	Muscle. (Excitability and contractility.)	
Aconitin . . . . .	2	min. 1	min. 3.1	min. 105	1/1000
" . . . . .	2	1.8	9.5	242	1/1300
" . . . . .	2	3	28	540	1/2600
Adrenalin . . . . .	4	112	1080	1260	1/5000
Alcohol . . . . .	4	90	372	480	1/50
Atropin . . . . .	6	7	20	90	1/100
Caffein . . . . .	4	20	60	900	1/1000
Chloroform . . . . .	2	1	3.5	30	1/1000
" . . . . .	1	15	40	205	1/5000
Cocaine . . . . .	3	5	52	960	1/1000
Curare . . . . .	4	31	45	960	1/500
Digitalin . . . . .	5	11	24	90	1/1000
Ether . . . . .	4	120	328	600	1/500
Nicotin . . . . .	4	94	265	1140	1/1000
Pilocarpin . . . . .	5	41	265	327	1/100
Quinine . . . . .	6	11	20	150	1/3 sat. sol.
Saponin . . . . .	3	31	39	210	1/800
Strychnin . . . . .	5	170	340	780	1/2000
Veratrin . . . . .	2	1	2	11	1/500,000
Ammonium chloride	5	1	7	17	6/10 n 1 + 1 serum
Calcium chloride .	4	90	150	211	6/10 n 1 + 1 serum
Magnesium chloride	4	8.5	26	82	6/10 n 1 + 1 serum
Potassium chloride.	2	0.5	12	180	6/10 n 1 + 4 serum
Cane sugar . . . . .	6	27	60	129	Molecular sol.
Glycerine . . . . .	5	3.5	10	85	Molecular sol.
Urea . . . . .	4	2	6	75	Molecular sol.



permeability, it is the permeability of these tissues themselves rather than that of accessory structures.

The three heart tissues in *Limulus* exhibit the same relative resistance to other injurious influences. An excised heart kept in serum or sea water at 10° C. to 12° C. dies after two or three days. The tissues die in the following order: ganglion, nerve plexus, muscle. In one experiment the ganglionic automatism ceased after thirty-two hours, the nerves failed to respond after thirty-eight hours, while the muscle responded for fifty-four hours. This bears out the assumption as to the rate of dying of the vertebrate heart tissues made by Carlson.<sup>4</sup> Moreover, the anterior, non-automatic region of the *Limulus* heart dies sooner than the posterior automatic region. In this regard the *Limulus* heart presents the same phenomenon as the vertebrate heart, the ventricle dying and going into rigor sooner than the auricles and the sinus venosus or the mouth of the large veins.<sup>5</sup>

The same relation obtains in regard to the paralysis of the heart tissues brought on by high and low temperatures. The reactions of the *Limulus* heart tissues to temperature variations have been investigated in detail by Carlson.<sup>6</sup> The ganglionic automatism is the first to cease, and the heart muscle fails to respond to the stimulation of the nerve plexus sooner than it fails to respond to direct stimulation. There is, moreover, a segmental or regional difference in regard to resistance to heat paralysis, the anterior or arterial end of the heart being paralyzed sooner or at lower temperature than the posterior venous end of the heart. It needs hardly be pointed out that we have here another striking similarity between the *Limulus* heart and the vertebrate heart, as it is well known that the auricles and sinus venosus continue in activity at a temperature that paralyzes the ventricle.<sup>7</sup>

So far, then, distilled water appears to be the only abnormal condition that effects paralysis in the heart muscle sooner than in the motor nerve plexus, as shown by Carlson's experiments.<sup>8</sup>

<sup>4</sup> CARLSON: This journal, 1907, xviii, p. 79.

<sup>5</sup> CARLSON: Zeitschrift für allgemeine Physiologie, 1904, iv, p. 269.

<sup>6</sup> CARLSON: This journal, 1906, xv, p. 207.

<sup>7</sup> STEWART: Journal of physiology, 1892, xiii, p. 59.

<sup>8</sup> CARLSON: This journal, 1906, xv, p. 112.

III. SUMMARY.

1. Alkaloids, anæsthetics, and chemicals in general produce paralysis of the *Limulus* heart tissues in the following order: (1) ganglion, (2) motor nerve plexus, (3) muscle.
2. The selective action of these drugs and chemicals on the three heart tissues is one of degree only.
3. In the reactions to heat and cold and in dying the *Limulus* heart exhibits the same differentiation between the venous and the aortic ends as the heart of vertebrates.

## THE RELATIVE HEMOLYTIC POWER OF SERUM AND LYMPH UNDER VARYING CONDITIONS OF LYMPH FORMATION.

BY W. T. HUGHES AND A. J. CARLSON.

*[From the Hull Physiological Laboratory of the University of Chicago.]*

THE experiments summarized in this report were undertaken with the view of securing further data on the relative composition of serum and lymph under different conditions of lymph formation. It seemed possible that some light may be thrown on the processes of lymph formation and the nature of the action of lymphagogues by determining the relative concentration of the hemolysins in serum and the lymph from different organs after intravenous injection of the lymphagogues.

But the question is not a simple one, mainly because of the fact that we do not yet know the place of formation of the various hemolysins. If they are formed by the tissue cells in general, they may reach the blood in the capillaries by absorption from the tissue lymph, or they may be absorbed by the lymphatic lymph and then reach the blood through the thoracic duct and the neck lymphatics. If they reach the blood by means of the lymphatics, they may accumulate there to a greater extent than in the lymph, because of the resistance offered to their re-entrance into the lymph by the capillary endothelium.

If the hemolysins are produced by the leucocytes, or by a certain class of leucocytes, they must be produced both in the blood and in the lymph. Their greater accumulation in the blood may then be due to their partial destruction in the lymph glands or to the resistance offered by the capillary endothelium to their passage into the tissue lymph and the lymph.

The difference in the hemolytic strength of the serum and the lymph may again be due to percentage variation either of the complement or the amboceptor, or, indeed, to percentage variations in

anti-lysins. Moreover, the lymphagogues may influence the rate of formation of the hemolysins, and thus effect a difference in their concentration without any variation in the possible interchange through capillary endothelium.

The first question to be determined, however, is whether lymphagogues such as peptone or albumoses tend to equalize the normal difference in hemolytic strength of serum and lymph. If these lymphagogues act, as Starling and others maintain, by injuring the capillary endothelium and thus increasing its permeability even to the serum proteids, we should expect a corresponding filtration of the lysins into the tissue lymph and the lymph.

#### I. THE LITERATURE.

Pagano<sup>1</sup> appears to have been the first man to investigate the relative hemolytic strength of serum and lymph of the same animal. He made use of the lysin for rabbits' corpuscles that exists in the normal serum of the dog, finding that the serum has a stronger action than lymph from the thoracic duct. Moreover, there is, according to Pagano, no difference in the hemolytic power of thoracic lymph during fasting and during digestion. The first point has been confirmed by Falloise<sup>2</sup> and by Batelli.<sup>3</sup> Batelli also found that the lymph from the thoracic duct has a stronger hemolytic action than the lymph from the neck lymphatics. The lytic strength of dog's serum and lymph on rabbits' corpuscles is given by Batelli as 11:7. Batelli argues that since the blood contains a greater number of large mononuclear leucocytes than does the lymph, these leucocytes are the producers of the lysins.

The hemolytic power of pericardial fluid, aqueous humor, and various pathological exudates and transudates has also been investigated with reference to that of the serum. According to Thioni,<sup>4</sup> the normal pericardial fluid of the ox contains amboceptor but no complement, and is therefore without hemolytic action. Gatti<sup>5</sup> found no hemolysins in the normal aqueous humor of the ox.

Strauss and Wolff<sup>6</sup> have attempted to correlate the hemolytic

<sup>1</sup> PAGANO: Archives italiennes de biologie, 1894, xx, p. 110.

<sup>2</sup> FALLOISE: Bulletin de académie royale de Belgique, 1903, No. 6.

<sup>3</sup> BATELLI: Comptes rendus de société biologique, 1904, lvi, p. 199.

<sup>4</sup> THIONI: Comptes rendus de société biologique, 1903, lv, p. 1592.

<sup>5</sup> GATTI: Cited from Biochemisches Centralblatt, 1905, iv, p. 678.

<sup>6</sup> STRAUSS and WOLFF: Fortschritte der Medizin, 1902, xx, p. 1.

strength of pathological exudates and transudates with their protein content. Marshall<sup>7</sup> found the hemolytic strength of ascites and pleurites fluid to be very variable. According to this observer the lytic action of these fluids on the blood corpuscles of other animals is stronger than that of the serum. The relation appears to be the same when only the complement content of these fluids and the serum is taken into consideration. Marshall does not seem to have made his comparison between these pathological fluids and the serum from the same patient. It is possible that the hemolytic power of the serum in pleurites is greater than normal. We have no reason for believing, however, that the relative hemolytic strength of normal serum and lymph in man is different from that in other mammals. Lüdke<sup>8</sup> reaches partly the same conclusion as Marshall regarding the hemolytic power of pathological body fluids of man. He finds the hemolytic strength very variable, and bearing no constant relation to their total protein content or to the hemolytic power of the serum.

Lüdke<sup>9</sup> has also demonstrated that the hemolysins may pass through even a double set of blood capillaries. On immunizing pregnant rabbits against ox corpuscles the foetal blood exhibited lytic action on ox corpuscles, but the lytic power of the blood of the foetus was less than that of the blood of the mother.

## II. METHODS.

The experiments were carried on under as nearly sterile conditions as possible. Sterile instruments were used in the operations, and the fluids were drawn through sterile cannulae into sterile vessels. In order to test whether or not sterile conditions influenced the results, a few experiments were carried on without these precautions. The result practically coincided with those carried on in the most careful manner.

In most cases the hemolytic tests were made on the day the fluids were drawn. In few cases the fluids were carried over in the ice chest till the next day. In order to determine whether or not this delay might influence the results, a sample lymph was carried over in the ice chest for nine days, without changing its hemolytic power.

<sup>7</sup> MARSHALL: *Journal of experimental medicine*, 1905, vi, p. 365.

<sup>8</sup> LÜDKE: *Centralblatt für Bakteriologie*, 1907, xlv, p. 268.

<sup>9</sup> LÜDKE: *Ibid.*, 1904, xxxvii, p. 288.

The dogs and cats were operated on under light ether anaesthesia, the horses under chloroform.

A series of five tubes for each serum or lymph were used in making the tests, and in each case control tubes containing a corpuscle suspension and physiological salt solution were subjected to exactly the same processes as the tubes containing the body fluids.

In the estimation of percentage of laking, the following scheme was adopted. One unit or 100 per cent laking consisted of the hemoglobin concentration of a 5 per cent suspension of fresh rabbits' corpuscles after complete water laking. For lesser degrees of laking fractions of this solution were diluted with water, making 90 per cent, 80 per cent, etc. A new standard of tints was made up for each new sample of blood corpuscle suspension used. The rabbits were very lightly anaesthetized when the blood was drawn for making the suspension.

The corpuscle suspension and the hemolytic fluids were usually mixed in the proportion of 1 to 0.5 and kept in the thermostat at 40° C. for from thirty to sixty minutes. The serum and lymph to be compared were always carried through simultaneously, so as to be subjected to exactly the same external conditions. In the case of the dogs immunized to typhoid bacilli whose serum exhibited a very strong laking action on rabbits' corpuscles tests were also made by adding smaller amounts of the sera to the corpuscle suspension, and readings made at shorter intervals than thirty minutes. But the observations recorded in Tables I and II were all made on mixture of corpuscle suspension and serum or lymph in the proportion of 1 to 0.5 and after thirty minutes' action at 40° C.

In the case of the lymphagogue lymph tests were made on successive samples collected up till one and one-half hours after the injection of the lymphagogues, so as to be sure that it was actual lymphagogue lymph and not simply the normal lymph formed prior to injection and simply forced out of the tissue by the fluid formed after the injection.

### III. RESULTS.

**I. Normal sera and lymphs.**—A considerable number of experiments were made to determine the normal range of variations in the lytic action on rabbits' corpuscles of normal serum and lymph of horse, cat, and dog. In the case of the horse the tests were made

TABLE I.  
THE RELATIVE HEMOLYTIC POWER ON RABBITS' CORPUSCLES OF NORMAL SERUM  
AND LYMPH OF HORSE, DOG, AND CAT.

No. of exp.	Animal.	Percentage of laking of rabbits' corpuscles in 60 minutes at 40° C.					
		Serum.	Thoracic lymph.	Neck lymph.	Lymph from forelimb.	Lymph from parotid gland.	Lymph from thyroid gland.
1	Horse	15	..	5	..	5	..
2	Horse	20	..	10	..	10	..
3	Horse	13	..	4	..	..	..
4	Horse	15	..	4	..	..	..
5	Horse	25	..	5	..	5	..
6	Horse	20	..	5	..	..	..
7	Horse	25	..	6	..	6	..
8	Horse	20	..	10	..	..	..
9	Dog	60	..	20	..	..	..
10	Dog	60	..	20	..	..	..
11	Dog	65	..	22	..	..	..
12	Dog	65	..	20	..	..	..
13	Dog	70	..	22	..	..	..
14	Dog	65	25	20	..	..	20
15	Dog	70	..	20	..	..	..
16	Dog	70	50	40	40	..	..
17	Dog	75	65	40	..	..	..
18	Dog	80	60	40	..	..	..
19	Dog	80	55	45	..	..	..
20 <sup>1</sup>	Dog	100	80	60	..	..	..
21 <sup>1</sup>	Dog	100	80	60	..	..	..
22 <sup>1</sup>	Dog	100	60	55	..	..	..
23 <sup>1</sup>	Dog	100	95	70	..	..	..
24 <sup>1</sup>	Dog	100	65	55	..	..	..
25	Cat	75	50	45	..	..	..
26	Cat	67	45	40	..	..	..

<sup>1</sup> Dogs 20 to 24 had been immunized by successive injections of twenty-four hours' broth culture of typhoid bacilli.



on sera and lymphs collected in the course of the investigation of the lymph formation in the salivary gland by Carlson, Greer, and Becht. The data of this first series are summarized in Table I. These data go to show that:

(1) The normal sera and lymph of horse, cat, and dog are lytic for rabbits' corpuscles.

(2) This normal non-specific hemolysin is more than twice as concentrated in the sera and lymph of dog and cat as in those of the horse.

(3) The sera and lymph of dogs previously rendered immune to typhoid bacilli exhibit a stronger lytic action on rabbits' corpuscles than do the normal serum and lymph.

(4) The lymph from the neck lymphatics, from the forelimbs, from the horse parotid gland before passing through any lymph gland, and from the dog's thyroid also before passing through any lymph gland, exhibit practically the same concentration of the hemolysin.

(5) The lymph from the thoracic duct exhibits invariably a stronger lytic action than the lymph from the limbs, the head and neck, the salivary glands and the thyroids.

(6) The concentration of this hemolytic substance appears, on the whole, to be subject to greater individual variations in the sera and lymph of the horse than in those of the cat and dog.

The greater lytic action of the body fluid of dogs immunized against typhoid bacilli was not looked for. The lymphs and serums of these animals were collected primarily for a different purpose. This greater hemolytic power may be due to a non-specific hemolytic action of the bacterio-lysins, or agglutinins, to an increase in the normal hemolysin or to the addition of another non-specific hemolysin as a side product of the processes of formation of the specific bacterio-lysins and precipitins.

The four samples of lymph from the parotid of the horse and the one sample from the thyroid of the dog were collected prior to passing through any lymph glands other than those that may possibly be situated within these organs themselves. The fact that these lymphs showed the same lytic power as the common neck lymph after having passed through the retro-pharyngeal lymph glands is significant from the point of view of the physiology of the lymph gland as well as in regard to the question of the place of formation of the hemolysin. In these cases the lysins could

only have come from glandular tissues or else been passed through the capillary endothelium from the blood. If the lysins are specific products of leucocytes, we should expect a stronger lytic action of the lymph after having sojourned in the large lymph gland, inasmuch as there is some evidence that the percentage of leucocytes in the lymph is increased in the lymph nodes.

2. *Lymphagogue lymphs.* — Our experiments on the lymphagogue lymph could not be carried to their completion at this time. Our results on this point should therefore be regarded as a preliminary statement only. In all six experiments were made with peptone or albumose solutions (10 per cent); one with extract of strawberry; four experiments with solutions of cane sugar (10 per cent); and one experiment with sodium chloride (5 per cent). The data are summarized in Table II. An examination of this table shows that:

(1) In the concentrations employed the cane sugar, sodium chloride, and strawberry extract produce no measurable change in the hemolytic power of the serum. Our results with peptone partly confirm the observations of Hewlett<sup>10</sup> and of Pfeiffer, that under certain conditions peptones diminish the hemolytic power of the blood. Hewlett investigated the relative lytic action of dog's serum and peptone blood on various foreign corpuscles, including those of the rabbit. Peptone administered intravenously in amounts up to 0.5 gm. per kilogram body weight have no appreciable effect on the lytic action of the dog's blood on rabbits' corpuscles, but in greater amounts the hemolytic power is decreased. Hewlett did not remove the corpuscles of the peptone blood, but the corpuscles probably have no specific action in augmenting or retarding the hemolysis by the serum. Pfeiffer<sup>11</sup> extended Hewlett's results to the blood of birds (chicken, goose). After injections of 0.5 gm. to 1 gm. peptone per kilogram body weight, varying degrees of decrease in the lytic power of the blood were noted. Peptone has no effect on the hemolysins of rabbits' blood immunized against foreign corpuscles.

In two of our experiments (5, 6) the peptone serum had a weaker lytic action than the normal serum. In both these cases the total quantity of peptone injected was 0.4 gm. per kilogram body weight.

<sup>10</sup> HEWLETT: *Archiv für experimentelle Pathologie und Pharmakologie*, 1903, xlix, p. 307.

<sup>11</sup> PFEIFFER: *Archiv für experimentelle Pathologie und Pharmakologie*, 1903, I, p. 158.

In our experiments the peptone solutions were injected at intervals of ten to twenty minutes, the total peptone injected in Nos. 1-4 varying from 0.15 to 0.3 gm. per kilogram body weight.

TABLE II.

THE RELATIVE HEMOLYTIC ACTION OF DOG'S AND CAT'S SERUM AND LYMPH ON RABBITS' CORPUSCLES BEFORE AND AFTER THE INJECTION OF VARIOUS LYMPHAGOGUES.

No. of exp.	Animal.	Lymphagogue.	Percentage of laking of rabbits' corpuscles in 30 minutes at 40° C.					
			Serum.		Thoracic lymph.		Neck lymph.	
			Normal.	Lymphagogue.	Normal.	Lymphagogue.	Normal.	Lymphagogue.
1	Dog	Peptone	70	70	50	50	40	40
2	Dog	Peptone	75	75	65	65	40	40
3	Dog	Peptone	80	80	60	65	40	40
4	Dog <sup>1</sup>	Peptone	100	100	80	90	60	60
5	Dog	Peptone	80	50	60	75	50	50
6	Dog	Peptone	70	60	50	55	35	35
7	Dog <sup>1</sup>	Strawberry	100	100	80	80	60	60
8	Dog	Cane sugar	80	80	55	55	45	45
9	Dog <sup>1</sup>	Cane sugar	100	100	60	60	55	55
10	Dog <sup>1</sup>	Cane sugar	100	100	95	100+	70	70
11	Cat	Cane sugar	80	80	50	50	45	45
12	Dog	Sodium chloride	100	100	65	65+	55	55

<sup>1</sup> Dog previously immunized by successive injections of twenty-four hours' broth culture of typhoid bacilli.

The sample of peptone blood was drawn about sixty minutes after the first injection. Peptone augments the flow of lymph from the thoracic duct, even in dilutions of 0.1 per kilogram body weight.

(2) There is no change in the hemolytic power of the neck lymph after the injection of these lymphagogues.

(3) In six out of the ten cases — Nos. 3, 4, 5, and 6 (peptone), No. 8 (cane sugar), and No. 10 (sodium chloride) — the thoracic

lymph exhibits a stronger hemolytic action after the lymphagogue injection.

(4) In one case out of the ten the lymphagogue lymph from the thoracic duct exhibits a stronger hemolytic action than the normal serum.

(5) In two cases the thoracic lymphagogue lymph has a stronger lytic power than the lymphagogue serum.

It has been shown by Starling that the increased lymph flow from the thoracic duct following the injection of peptone into the blood comes mainly from the liver, and that this lymph is richer in protein than the normal. Starling's interpretation of the nature of this lymphagogue action is that the peptone by injuring the capillary endothelium in the liver increases its permeability, and the result is an augmented filtration of a more concentrated liquid from the blood. Neither the injury hypothesis nor the stimulation hypothesis suffice to explain the fact that the peptone action is practically confined to one organ alone, namely, the liver. But the observations of Timofejewsky<sup>12</sup> indicate that this increase in the protein content is not in every case such as would result from an augmented filtration from the blood through injured capillary walls. For example, of the total proteins in the serum and the lymph, globulin has a higher percentage in the former than in the latter. Peptone injection does not alter this ratio of globulins to total proteins in serum and lymph, although it increases the total lymph protein. Peptone also increases the percentage of the globulin, but this is done to the same degree in the serum and in the blood.

In all our experiments the peptone injections were followed by a greatly augmented lymph flow from the thoracic duct. On Starling's hypothesis this lymphagogue lymph should exhibit a stronger hemolytic action than the normal, because the blood contains the lysis in a greater concentration, and if peptone injures the capillary walls in such a way as to allow a more rapid filtration of the serum proteids, the same must be true, even in a greater degree, for the hemolysins. In two out of the six experiments no increase was obtained, while in Experiments 5 and 6 the normal relation between serum and thoracic lymph was reversed by the peptone injection. This fact goes to show that we are dealing with more complicated processes than mechanical filtration.

The mechanical explanation of the mechanism of action of the

<sup>12</sup> TIMOFEJEWSKY: *Zeitschrift für Biologie*, 1899, xxxviii, p. 618.

crystalloid lymphagogues is that of increased capillary and therefore filtration pressure by hydremic plethora. There is no evidence of injury to the capillary walls with attendant greater permeability. As the hydremic plethora is produced by withdrawal of water from the tissues, the blood becomes necessarily more dilute as regards its organic constituents. In fact, the injection of the crystalloid solution itself diminishes the concentration of the organic constituents in the blood. According to the mechanical theory, lymph following injection of the crystalloid lymphagogues must be poorer than the normal in organic constituents. In our experiments this would be equivalent to a diminution of its hemolytic power. There is no such diminution, but, on the contrary, a slight increase in the lytic strength of the thoracic lymph in Experiments 8 and 10.

Both the cane sugar and the sodium chloride increase the lymph flow from the thoracic and neck ducts, but this increase is not marked in the case of the latter. Inasmuch as these lymphagogues do not alter the concentration of the hemolysin in the blood, it is obvious that an increased formation of the hemolysin or a progressive destruction of anti-lysins must take place in the tissues or the lymph in order to maintain the concentration in the lymphagogue lymph. The mechanism of this co-ordination requires further investigation.

In one case (8) the thoracic lymphagogue lymph exhibits a greater hemolytic action than the normal serum. In this animal, however, the normal thoracic lymph had nearly as strong action as the normal serum. In case the lymphagogue serum had shown less hemolytic power than the normal serum this state of things might have come about by an inhibition of the lysin by the sugar. As that is not the case, we must have an augmented rate of formation of the hemolysin in the tissues in such a way that they reach the thoracic lymph stream.

3. **Other body fluids.** — In some cases the normal pericardial fluid of the dog has a very slight hemolytic action on rabbits' corpuscles. Thus, out of twelve dogs tested six showed no hemolytic action whatever, while the remaining six had a slight action. No examinations of the fluid for leucocytes were made in the case of the fluid giving positive results. All the samples of the pericardial fluid were secured under the same condition. There was no contamination with serum or lymph in the case of the fluids showing hemolysis.

The aqueous humor of the dog presents similar variations in the lytic power on rabbits' corpuscles. Out of thirteen experiments eight were entirely negative, while the remaining five gave positive results. But the hemolytic power even in these cases was less than 1 per cent of that of the neck lymph.

Tests were made on the cerebro-spinal fluid of fifteen dogs with negative results in each case. No laking of rabbits' corpuscles occurs in the normal cerebro-spinal fluid of the dog.

The work of Cavazzani, Capelletti, Pettit, and Girard, and Meek<sup>13</sup> seems to show that the cerebro-spinal fluid is formed, at least in part, by secretory activity of the epithelial cells of the choroid plexus. The quantity of the fluid is not affected by lymphagogues, for example, while it is increased by pilocarpin and checked by atropin. After pilocarpin the cells exhibit the typical changes of active gland cells.

These data do not permit any conclusions regarding the place of formation of the hemolysins or the nature of the processes of formation of the pericardial, the aqueous, and the cerebro-spinal fluid, at least till we have determined whether the negative results are due to the absence of the lysins or the presence of anti-lysins.

#### IV. SUMMARY AND CONCLUSIONS.

1. The concentration of the hemolysins for rabbits' corpuscles present in the normal body fluids of the dog, the cat, and the horse exhibit the following descending series: serum; lymph from the thoracic duct; neck lymph, lymph from the limbs, the thyroids, and the salivary glands; pericardial fluid; aqueous humor. The cerebro-spinal fluid does not contain any of these lysins.

2. The large retro-pharyngeal lymph glands do not appear to influence the concentration of the hemolysins in the lymph passing through them.

3. Immunization against the typhoid bacilli appears to increase the hemolytic power of the serum and the lymph in the dog.

4. The lymphagogues do not alter the hemolytic power of the neck lymph. Peptone, hypertonic cane sugar, and sodium chloride may increase the hemolytic power of the lymph from the thoracic duct. In the case of the peptone this augmentation of the lytic

<sup>13</sup> The literature discussed by MEEK: *Journal of comparative neurology*, 1907, xvii, p. 286.

power of the thoracic lymph may take place *pari passu* with a decrease in that of the serum, so that the normal relations become reversed, the thoracic lymph having a stronger lytic action than the serum.

5. The mechanical explanation of the action of these lymphagogues does not suffice to account for our results. Peptone lymph from the thoracic duct may exhibit the same lytic power as normal lymph, or it may exhibit a stronger lytic action than the normal lymph, while that of the serum has at the same time diminished below that of the normal lymph. The primary point of action of the peptone is probably the liver cells. The diminished lytic power of the peptone serum may be due to anti-lysins produced in the liver and passed directly into the blood capillaries from the tissue lymph. On the injury hypothesis of the peptone action they should pass just as readily into the lymph proper.

6. There is some evidence that these lymphagogues may augment the rate of production of these hemolysins, but this phase of the question requires further study.



## THE COMPRESSIBILITIES OF GELATINE SOLUTIONS AND OF MUSCLE.

BY LAWRENCE J. HENDERSON AND F. N. BRINK.

[From the Chemical Laboratory of Harvard College.]

**C**OMPRESSIBILITY is a property which, so far as we can discover, has never been quantitatively studied, either in protoplasm or in colloidal solutions. Apart from the desirability of recording all possible physical properties of protoplasm, the determination of its compressibility, as well as the determination of the compressibility of colloidal solutions, is not without interest, because it is directly important in the understanding of the nature of colloids and of protoplasm. Accordingly we have been glad to avail ourselves of the opportunity kindly presented to us by Professor T. W. Richards of determining this property for gelatine solutions and for perfectly fresh rabbit's muscle with the aid of the compression apparatus now in use in the Chemical Laboratory of Harvard College.<sup>1</sup>

**The compression apparatus.**—The compression was carried on by means of a Cailletet compression pump of lever-and-screw type, filled with oil and communicating through a stout copper tube with a heavy steel barrel of suitable size to contain the compression jacket; upon the steel barrel there fitted a steel screw cap. Connected with the apparatus was a hydraulic gauge for registering the pressure in kilograms per square centimetre.

The glass jacket (Fig. 1), containing gelatine solution, or muscle suspended in normal saline solution, was suspended in oil and mercury within the steel barrel, hanging down from the screw cap.

<sup>1</sup> For a detailed description of this apparatus, see RICHARDS, STULL, BRINK, and BONNET, The compressibility of the elements and their periodic relations, Carnegie Institution of Washington, Publication No. 76, May, 1907, p. 9; also *Zeitschrift für physikalische Chemie*, 1907, lxi, pp. 77, 100, 171, 183. We wish to express our thanks to the Carnegie Institution of Washington for the use of the apparatus.

The whole of the barrel below the screw cap was immersed in the water of a thermostat, thereby keeping the temperature constant within three one-thousandths of a degree.

**Method.** — In carrying out the determinations the procedure described in Richards's papers<sup>2</sup> was closely followed, the glass jacket being filled with the substance to be compressed, gelatine solution, or muscle suspended in normal saline solution, and the capillary U tube filled with mercury in such amount that upon exerting a



FIGURE 1. — Compression tube.

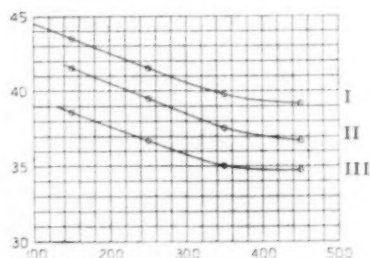


FIGURE 2. — The ordinates show compressibility  $\times 10^{-6}$ . The abscissas show pressure in megabars. Curve I, compressibility of 0.2 per cent gelatine solution. Curve II, compressibility of 10 per cent gelatine solution. Curve III, compressibility of rabbit's muscle.

pressure of from 50 to 100 atmospheres the electrical contact between the platinum point and the mercury was broken. Thereafter small weighed quantities of mercury were added to that already in the apparatus, and after each addition the pressure at which the electrical contact broke was noted; finally a part of the mercury was removed, and a reading near the original one was obtained. From the data thus found curves were plotted with pressures as abscissas and weights of mercury added as ordinates. Together with these curves was plotted a curve obtained in precisely the same way when the whole jacket was filled with mercury. From these curves the compressibilities were calculated according to the formula<sup>3</sup>

<sup>2</sup> RICHARDS: *Loc. cit.*

<sup>3</sup> The formula in the case of muscle is a little more complicated as a result of the presence of normal saline solution, for the compressibility of which correction has to be applied. For a full discussion of the calculation, consult RICHARDS: *Loc. cit.*, pp. 21, 36, and 59.

$$\beta = \frac{M \times S}{13.55 \times P \times W} + X,$$

where  $M$  represents the difference between the weight of mercury on the gelatine curve and the weight of mercury on the mercury curve for the interval of pressure,  $P$ ,  $S$  being the specific gravity of the gelatine solution,  $W$  the weight of the gelatine used; 13.55 being the specific gravity of mercury,  $X$  the compressibility of mercury for the interval expressed by  $P$ , and  $\beta$  the desired average compressibility, that is to say, the average change of volume caused by 1 kilogram per square centimetre pressure within the range of pressure expressed by  $P$ . For further details regarding the method, see the publication above referred to. All the determinations in this paper were carried out under precisely the same conditions and in precisely the same way as were the compressibilities determined by one of us (B.), of solids suspended in water.

*Compressibility of a 10 per cent gelatine solution.*—Weight of gelatine solution = 10.083 gm. Specific gravity of solution = 1.03.

## OBSERVED VALUES.

Hg added.	Pressure Kg/cm <sup>2</sup> .
0.000	59
0.229	103
0.760	207
1.318	324
1.884	442
2.208	515
0.215	100

The following table is taken directly from the plotted curves:

## DEDUCED VALUES.

Pressure Kg/cm <sup>2</sup> .	Hg Weight.	I Difference.	II Hg correction.	I-II M.
100	0.215	0.515	0.024	0.491
200	0.730	0.490	0.024	0.466
300	1.220	0.463	0.024	0.439
400	1.683	0.457	0.024	0.433
500	2.140			
100-500	....	1.925	0.096	1.829

With the aid of the equation

$$\beta = \frac{M \times S}{13.55 \times P \times w} + X,$$

the calculations are carried out as follows:

100-200 Kg/cm<sup>2</sup>

$$\frac{0.491 \times 1.03}{13.55 \times 100 \times 10.08} + 0.00000375 = 0.000041$$

200-300 Kg/cm<sup>2</sup>

$$\frac{0.466 \times 1.03}{13.55 \times 100 \times 10.08} + 0.00000372 = 0.000039$$

300-400 Kg/cm<sup>2</sup>

$$\frac{0.439 \times 1.03}{13.55 \times 100 \times 10.08} + 0.00000369 = 0.000037$$

400-500 Kg/cm<sup>2</sup>

$$\frac{0.433 \times 1.03}{13.55 \times 100 \times 10.08} + 0.00000364 = 0.000036$$

100-500 Kg/cm<sup>2</sup>

$$\frac{1.829 \times 1.03}{13.55 \times 400 \times 10.08} + 0.0000037 = 0.000038$$

*Compressibility of a 0.2 per cent gelatine solution.* — The specific gravity of this solution must be very near 1.00; it cannot be less than 1 nor greater than 1.03, hence its determination was not necessary. Weight of gelatine solution = 9.786 gm.

## OBSERVED VALUES.

Hg added.	Pressure.
0.000	28
0.419	103
1.036	208
1.610	332
2.065	424
2.550	525
0.457	110

## DEDUCED VALUES.

Pressure.	Hg Weight.	I Difference.	II Hg correction.	I-II M.
100	0.400	. . . 0.540	0.024	0.516
200	0.940	. . . 0.513	0.024	0.489
300	1.453	. . . 0.492	0.024	0.468
400	1.945	. . . 0.483	0.024	0.459
500	2.428			
100-500	....	2.028	0.096	1.932

The compressibilities calculated from these values are as follows:

Pressure interval.	Compressibility.
100-200 . . . . .	0.000043
200-300 . . . . .	0.000041
300-400 . . . . .	0.000039
400-500 . . . . .	0.000038
100-500 . . . . .	0.000040

*The behavior of muscle under pressure.*—A rabbit was killed by bleeding, and four small muscles were carefully removed from the forelegs, taking pains to preserve them intact and free them from other material so far as might be. These muscles were then introduced into the compression apparatus, the interstices were filled with normal saline solution, and the compressibility of the system was determined. The compressibility of the saline solution and its specific gravity were assumed to be equal to the compressibility and specific gravity of water, an assumption which cannot affect the significant figures in the result. Any gas which may have been present must have been forced into solution long before the first reading was taken.

Specific gravity of muscle = 1.06.<sup>4</sup> Weight of muscle = 8.64 gm. Weight of salt solution = 1.72.

## OBSERVED VALUES.

Hg added.	Pressure.
0.000	86
0.564	200
1.122	320
1.707	450
2.011	518
0.228	110

## DEDUCED VALUES.

Kg/cm <sup>2</sup> Pressure.	Hg Weight.	I Difference.	II Hg correction.	I-II M.
100	0.070	. . . 0.495	0.024	0.471
200	0.565	. . . 0.470	0.024	0.446
300	1.035	. . . 0.450	0.024	0.426
400	1.485	. . . 0.445	0.024	0.421
500	1.930			
100-500	...	1.860	0.096	1.764

<sup>4</sup> HERMANN: *Handbuch der Physiologie*, i, part I, p. 13.

From these values the following compressibilities are calculated:

Pressure interval.	Compressibility.
100-200 . . . . .	0.000038
200-300 . . . . .	0.000036
300-400 . . . . .	0.000034
400-500 . . . . .	0.000034
100-500 . . . . .	0.0000355

Duplication of the experiments was not considered necessary, for in carrying out the determinations the addition of successive small amounts of mercury, followed by the removal of an amount approximately equal to all the mercury which had been added, amounts to a multiple reduplication of the work.<sup>5</sup>

The outcome of these experiments may be represented by the following table:

Substance.	Average compressibility ( <i>i. e.</i> , average change of volume caused by 1 megabar <sup>6</sup> pressure) between 100 and 500 megabars $\times 10^6$ .
Muscle . . . . .	36
Ten per cent gelatine solution . . . . .	39
Two-tenths per cent gelatine solution . . . . .	41

The results are graphically represented on the accompanying diagram.

For comparison the following compressibilities, determined in this laboratory, are cited:

Substance.	Average compressibility.
Water . . . . .	42.
Silicon . . . . .	0.16
Copper * . . . . .	0.54
Mercury <sup>7</sup> . . . . .	3.7 <sup>1</sup>
Potassium . . . . .	31.5

Above 50 megabars<sup>7</sup> (atmospheres) pressure the compressibilities of gelatine solutions and muscle vary in a regular manner, very much as the compressibilities of simple liquids and solids vary.

<sup>5</sup> RICHARDS: *Loc. cit.*, p. 62.

<sup>6</sup> One megabar is the pressure of 1 megadyne per square centimetre; it equals 0.987 atmosphere. Calculated for kilograms per square centimetre pressure, these values are 35, 38, and 40, respectively, for atmospheres 37, 39, and 41, respectively.

<sup>7</sup> Standard.

Moreover the compressibilities of these colloidal systems are probably of the same magnitude as the compressibilities of simple aqueous solutions of like concentration; indeed the compressibility of the more dilute gelatine solution is nearly equal to that of water.

The results of these experiments indicate that colloidal solutions and protoplasm are probably just as closely packed aggregates as are simple solutions. A further interpretation of the results in terms of the current theories of colloids seems unprofitable because those theories are not yet precise.

The present investigation offers no evidence regarding the compressibility of the substances employed for pressures below about 50 megabars. It is, of course, probable that the compressibilities do not vary greatly between 1 and 50 megabars; at present, however, that cannot be said with absolute certainty.

It is a pleasure to record our indebtedness to the Elizabeth Thompson Science Fund for aid in this investigation.

#### SUMMARY.

The compressibilities of gelatine solutions and of muscle have been accurately measured. They are found to be somewhat less than the compressibility of water, and are lower the more concentrated the solution. With change in pressure they vary in much the same way that the compressibilities of simple substances vary.



## THE SENSORY NERVES OF THE HEART AND BLOOD VESSELS AS A FACTOR IN DETERMINING THE ACTION OF DRUGS.

BY D. E. JACKSON AND S. A. MATTHEWS.

[From the Laboratories of Biochemistry, Pharmacology, and Experimental Therapeutics of the University of Chicago.]

IT has long been known that one of the earlier symptoms of aconite poisoning in mammals consists in a well-defined retardation of the pulse rate. This is usually accompanied by a marked and persistent fall in the blood pressure. Achscharumow<sup>1</sup> early attributed the slowing of the heart to a direct stimulation of the inhibitory centre in the medulla-oblongata. In recent years practically all investigators have ascribed the fall in blood pressure to the accompanying retardation of the cardiac rhythm. (This, of course, refers only to the earlier action of the drug, and not to the later stages in which the heart beat finally becomes very rapid and at last extremely irregular.)

These factors are undoubtedly sufficient to account for a very considerable decrease in the general arterial tension. But, as has been pointed out by Mathews,<sup>2</sup> the primary fall in pressure often seems to be much greater than could be expected from the cardiac slowing present in the given case. This condition is often especially well marked in cats.

That the vaso-motor centre is not appreciably affected early in the stage of decreased pressure is well shown by the fact that it can be readily excited reflexly by electrical stimulation of a sensory nerve. It has also been demonstrated that the peripheral endings of the vaso-constrictors (splanchnics) are still fully capable of carrying out their normal function at a very late stage of the intoxication.<sup>3</sup>

<sup>1</sup> ACHSCHARUMOW : *Archiv für Anatomie und Physiologie*, 1866.

<sup>2</sup> PROFESSOR A. P. MATHEWS : Unpublished observation, 1907.

<sup>3</sup> CASH and DUNSTAN : *Philosophical transactions of the Royal Society of London, Series B.*, 1898, cxc, p. 258.

It is also evident that the vessel walls themselves cannot be noticeably weakened during the earlier stages of the poisoning. That the heart is not depressed is shown by the fact that in dogs, *e. g.*, section of the vagi or atropin at once restores the normal beat.

One of the earliest and most characteristic actions of aconitine is a marked and extensive stimulation of peripheral sensory nerve endings. In consideration of these facts it was suggested<sup>4</sup> that aconitine might indirectly (through the inhibitory and vaso-dilator centres) effect a fall in blood pressure by stimulation of the cardiac endings of the depressor nerve. The following experiments were undertaken with the view of demonstrating whether or not such action occurs.

Unfortunately, of the animals usually available for experimentation, the rabbit is the only one in which it is possible to isolate the depressor nerves. We have accordingly done most work upon these. The rabbits were given urethane and ether, or ether alone, and prepared in the usual manner for taking blood-pressure tracings. The depressor nerves (usually vagi and sympathetic also) were isolated, and arranged for immediate section at any time desired.

Aconitine (1/40 per cent) was then very cautiously injected intravenously, the blood pressure meanwhile being very carefully observed. A small injection (*e. g.*, 1/4 to 1/2 c.c.) was usually sufficient to induce a noticeable fall in pressure. The drug was then slowly administered until the vagus action became very strong. The depressor nerves were then cut. The result was usually a very perceptible rise in blood pressure. It was also observed, in some instances, that when the blood pressure had become irregular before the depressors were cut, then section of these fibres caused the arterial tension to become perfectly regular again. The slowing of the heart due to central vagus stimulation was not, however, appreciably affected. Unfortunately, rabbits were rather unsatisfactory subjects for such experiments, owing to the fact that in these animals stimulation of the depressor nerves causes but little reflex vagus slowing of the heart. The respiratory effects of the aconite also come on very early, and in many instances it was impossible to follow the action of the drug. Aside from these difficulties, however, it seemed quite evident that in most cases, at least, section of the depressors favored the recovery of the animal, as larger doses were then required to produce death.

<sup>4</sup> PROFESSOR A. P. MATHEWS: Unpublished, 1907.

It has been repeatedly observed that section of the vagi may recover an animal from a dose of aconite which would otherwise prove fatal. The result in these cases is an immediate acceleration of the heart and a rise in blood pressure. Most of these experiments have undoubtedly been performed upon cats and dogs, in which animals the depressor fibres are included in the vagus trunks. In these cases, of course, it is difficult to say how much, or if any, of the fall in pressure and slowing of the heart ordinarily produced is due to stimulation of the depressor endings, for the rise in pressure in an animal whose vagus nerves have been sectioned before the aconite was administered would seem to indicate a direct stimulation of the vaso-constrictor centre, although it might, of course, be due to reflex excitation of other vaso-sensory endings than those of the heart.

The peculiar action of aconite on the blood pressure in some instances has, indeed, led us to believe that it may also possibly bring into action some other form of reflex vaso-constriction by stimulation of some afferent endings in the lumen of the vessels themselves. We have repeatedly observed evidences in this direction in the case of other irritant substances also; *e. g.*, if a fairly strong solution of alcohol, digitalis, etc., is injected into the femoral vein, there is often observed a small immediate rise in pressure, followed at an interval by the general action of the drug. In many cases this rise appears rather too soon for the drug to have reached the medullary centres, and it seems improbable that a direct action on the musculature of the heart or vessels should account for all cases. That it is not due to a sudden increase in the volume of the vascular contents is apparently disproved by the facts that it may often be obtained by extremely small injections, and also that with many drugs no rise is produced. We have also often seen a slight rise in pressure produced by electrical stimulation of a carefully isolated portion of the jugular vein (in rabbits). The difficulty, however, of making exact and entirely satisfactory experimental observations in this direction is perfectly obvious.

It was observed by Brunton and Cash,<sup>5</sup> and later verified by Cash and Dunstan,<sup>6</sup> that if an animal be placed in an atmosphere warmer than its normal body temperature and then be given aconite, its temperature will rise more rapidly than normally. And further, if

<sup>5</sup> BRUNTON and CASH: *St. Bartholomew Hospital reports*, 1886, xxii, p. 271.

<sup>6</sup> CASH and DUNSTAN: *Loc. cit.* p. 264.

the animal be placed in a cold bath, its temperature will fall more rapidly than in a normal animal. Circulatory disturbances, especially dilatation of the cutaneous vessels, are perhaps sufficient to account for these observations. The abnormal thermometric variations have, however, been attributed to a direct action of the aconite upon the central heat-regulating mechanism. At ordinary room temperatures the temperature of an animal treated with a moderate dose of aconite will at first rise a little above the normal, and later fall to slightly below the normal. The strong primary stimulation and later paralysis of the peripheral sensory endings have suggested to us the possibility that the regulation of the general temperature may in some measure be reflexly influenced by the action of the drug upon the peripheral endings of those nerves which ordinarily report sensations of heat and cold.<sup>7</sup> In this connection may be mentioned the chilly sensation<sup>8</sup> which in aconite poisoning occurs before either the general temperature or the cutaneous circulation have been materially affected, but at a time when the drug has certainly acted upon a number of peripheral sensory endings. Again, exact experimental observations are not readily obtained. The importance which an action of this kind may have in other relations has led us to believe that the suggestion may be worth recording.

The foregoing observations on the action of the depressor in aconite poisoning show clearly the importance of sensory nerve terminations in the heart and blood vessels in determining the action of drugs. Further work in this direction is being carried on.

In closing we wish to thank Professor A. P. Mathews for suggesting the line of work taken up in this paper.

<sup>7</sup> TIGERSTEDT: Text-book of human physiology, 1906, p. 408.

<sup>8</sup> SOLLMANN: Text-book of pharmacology, 1906, p. 318.

# ON A SERIES OF FEEDING AND INJECTION EXPERIMENTS FOLLOWING THE ESTABLISHMENT OF THE ECK FISTULA IN DOGS.

By P. B. HAWK.

[From the Laboratory of Physiological Chemistry of the Department of Medicine of the University of Pennsylvania.]

## CONTENTS.

	Page
I. Introduction . . . . .	260
II. Description . . . . .	262
III. Experimental data . . . . .	263
1. Experiment I . . . . .	263
(a) Operation and preliminary feeding . . . . .	263
(b) Toxic symptoms . . . . .	264
(c) Meat-free diet . . . . .	267
(d) Post-mortem examination . . . . .	267
2. Experiment II . . . . .	268
(a) Operation and preliminary feeding . . . . .	268
(b) Feeding of Liebig's extract . . . . .	268
(c) Toxic symptoms . . . . .	269
(d) Post-mortem examination . . . . .	270
3. Experiment III . . . . .	270
(a) Operation and preliminary feeding . . . . .	270
(b) Injection of sodium carbamate and feeding of Liebig's extract and sodium carbamate . . . . .	270
(c) Meat-free diet plus Liebig's extract . . . . .	271
(d) Meat diet plus Liebig's extract . . . . .	272
(e) Post-mortem examination . . . . .	273
4. Experiment IV . . . . .	273
(a) Operation and preliminary feeding . . . . .	273
(b) Meat diet . . . . .	274
(c) Post-mortem examination . . . . .	274
5. Experiment V . . . . .	275
(a) Operation and feeding tests . . . . .	275
(b) Post-mortem examination . . . . .	276
6. Experiment VI . . . . .	276
(a) Operation and preliminary feeding . . . . .	276
(b) Meat diet . . . . .	276
(c) Exploratory operation . . . . .	276
(d) Meat diet . . . . .	277
(e) Post-mortem examination . . . . .	277

IV. Control experiments on normal dogs . . . . .	277
1. Injection of sodium carbamate . . . . .	277
2. Feeding of sodium carbamate . . . . .	278
3. Injection of Liebig's extract . . . . .	278
4. Feeding of Liebig's extract . . . . .	280
V. Conclusions . . . . .	280

## I. INTRODUCTION.

THE origin of the Eck fistula dates from 1877, in which year the first fistula of this character was established by von Eck.<sup>1</sup> Five years later Stolnikow<sup>2</sup> repeated Eck's operation with satisfactory results. Both of these investigators used dogs as subjects, but neither of them made any reliable metabolism experiments with the animals operated upon. Several years later, however, Hahn, Massen, Nencki, and Pawlow<sup>3</sup> published data from an elaborate series of investigations upon Eck-fistula dogs. In this series of studies Pawlow and Massen established the fistulas in dogs which were subsequently subjected to metabolism studies by Nencki and Hahn. One of the most striking things observed by Pawlow and his associates in connection with this elaborate series of tests was the fact that the ingestion of meat by a dog on which Eck's operation had been performed was followed sooner or later by a series of toxic symptoms in the course of which the animals became anæsthetic, ataxic, and cataleptic, and suffered the loss of sight and hearing. Generally the symptoms terminated fatally, but occasionally the animal recovered. The post-mortem examination of such animals as exhibited no such symptoms as those described above indicated that the fistula opening was exceedingly small and that a collateral circulation had been established. The chemical examination of the urine of such dogs as showed the toxic symptoms generally indicated a relative decrease in the output of urea and a relative increase in the content of uric acid and ammonia. Such urines further contained carbamic acid, this substance being present in greatest amount in the urine voided during or immediately following the occurrence of the toxic symptoms. These investigators later injected sodium carbamate into normal dogs and obtained symptoms similar to those produced in the Eck-

<sup>1</sup> ECK: *Militär-medicinischer Journal*, 1877, cxxxii.

<sup>2</sup> STOLNIKOW: *Archiv für die gesammte Physiologie*, 1882, xxviii, p. 255.

<sup>3</sup> HAHN, MASSEN, NENCKI, and PAWLOW: *Archiv für experimentelle Pathologie und Pharmacologie*, 1893, xxxii, p. 161.

fistula animals after meat feeding. The feeding of the carbamate to Eck-fistula dogs was also followed by the customary toxic symptoms. From these data the investigators formulated the theory that the toxic symptoms observed in the Eck-fistula animals after meat feeding were due to the influence of the carbamic acid.

Later Nencki, Pawlow, and Zaleski<sup>4</sup> conducted a series of investigations, as the result of which they proposed the theory that the toxic symptoms observed were due to ammonia poisoning and not to carbamic acid poisoning. These experiments were followed by still other experiments by Nencki and Pawlow<sup>5</sup> in which the establishment of the Eck fistula was accompanied by the removal of a large part of the liver in some instances and in others by the ligation of the hepatic artery. They concluded from these experiments that the ammonia intoxication was not sufficient to have caused the toxic symptoms observed. The animals invariably died a few hours after the operation.

Others who have made important contributions to the Eck-fistula literature are Lieblein,<sup>6</sup> Salaskin,<sup>7</sup> Rothberger and Winterberg,<sup>8</sup> and Macleod.<sup>9</sup> Lieblein destroyed the liver cells by injecting acid into the hepatic ducts, after which the animals exhibited symptoms similar to those observed after the extirpation of the liver. These symptoms were probably not due to ammonia intoxication under these conditions. Salaskin, as a result of his investigations on Eck-fistula dogs, came to the conclusion that ammonia was the prime factor in causing the nervous disturbances observed. Rothberger and Winterberg, from a series of experiments made on a large number of dogs, presented the theory that the toxic symptoms were due to the presence of some toxic substance which the liver normally destroys; they did not believe carbamic acid to be the cause of the trouble. Macleod in his investigations confirmed in general

<sup>4</sup> NENCKI, PAWLOW, and ZALESKI: *Archiv für experimentelle Pathologie und Pharmacologie*, 1896, xxxvii, p. 26.

<sup>5</sup> NENCKI and PAWLOW: *Archiv für experimentelle Pathologie und Pharmacologie*, 1897, xxxviii, p. 215.

<sup>6</sup> LIEBLEIN: *Archiv für experimentelle Pathologie und Pharmacologie*, 1894, xxxiii, p. 318.

<sup>7</sup> SALASKIN: *Zeitschrift für physiologische Chemie*, 1898, xxv, p. 449.

<sup>8</sup> ROTHBERGER and WINTERBERG: *Zeitschrift für experimentelle Pathologie und Therapie*, 1905, i, pp. 312-359.

<sup>9</sup> MACLEOD: *Studies in pathology*, written by Alumni to celebrate the Quatercentenary of the University of Aberdeen, Aberdeen, 1906, p. 267.



the findings of Hahn, Massen, Nencki, and Pawlow, but is apparently not inclined to accept the theory that carbamic acid is the origin of the toxic symptoms. In his chemical examination of the urine nothing abnormal was noted except the presence of a large amount of albumin in the urine of an Eck-fistula dog on the day following the addition of meat extract to the diet. This investigator emphasizes the belief that, in order to obtain the most reliable data regarding the cause of the toxic symptoms observed in dogs after the establishment of the Eck fistula, the urinary analysis must be supplemented or even replaced by the collection of extensive data from the analysis of the blood and organs. Macleod's animals were operated on by Herrick.<sup>10</sup>

## II. DESCRIPTION.

The investigation consisted of a series of feeding and injection experiments upon dogs in which the liver function was greatly impaired by the successful performance of Eck's operation. A preliminary report on some of the experiments has already been made.<sup>11</sup> The operations were in each instance performed by Dr. J. E. Sweet<sup>12</sup> of the University of Pennsylvania. The author wishes to express his great appreciation of the courtesy extended to him by Dr. Sweet in permitting him to use the dogs for metabolism studies, as well as for his kindness in making the post-mortem examinations. Instead of employing scissors to make the openings upon which the intercommunication between the portal vein and the vena cava was dependent, as had been the custom of previous operators, Sweet devised a very ingenious method which embraced the use of an electric cautery for this purpose. This device, supplemented by his brilliant technique, was responsible for the excellent results he obtained. Six dogs in which the Eck fistula had been established were used as subjects in the course of the investigation, the length of the experimental periods varying from twenty-eight days to one hundred and thirty-five days for the different dogs, and the aggregate number of days being four hundred and eleven. Two or three animals were frequently under

<sup>10</sup> HERRICK: *Journal of experimental medicine*, 1905, vii, p. 751.

<sup>11</sup> HAWK: *Proceedings of the American Physiological Society*, Dec. 27-28, 1904; *This journal*, 1905, xiii, p. xiv.

<sup>12</sup> SWEET: *Journal of experimental medicine*, 1905, vii, p. 161.

experimentation simultaneously, the actual number of days during which the feeding experiments on the Eck-fistula dogs were carried out being two hundred and thirty-one. In addition to these experiments nine feeding and injection experiments were made on four normal control animals.

### III. EXPERIMENTAL DATA.

1. **Experiment I.** *a. Operation and preliminary feeding.* — The subject of this experiment (Dog No. 2) was a bitch weighing 9.64 kg., Eck's operation being performed upon the animal on February 16. For six days following the operation the animal took practically no food, but drank about 300 c.c. of water each day, a portion of which was subsequently vomited. On the seventh day of the experiment (February 23) the animal was induced to drink a little milk, most of which was retained. From February 23 to February 26 the milk diet was continued, and all signs of vomiting were absent. On February 27 the dog was fed meat for the first time, her diet for this day consisting of 45 gm. of beef meal and 150 c.c. of milk. The milk and beef meal were mixed, and fed by means of a stomach tube,<sup>13</sup> since the animal would not voluntarily eat the beef meal. On the next day a mixture of 20 gm. of meal and 300 c.c. of milk was fed in the same way, whereas on February 29 the ration was increased to 80 gm. of meal and 400 c.c. of milk.

Up to this time the feeding of the meat had been the cause of no disturbances, so far as we could judge, and inasmuch as we were anxious to ascertain what the effect of the ingestion of an excessive amount of meat would be, we fed the animal 150 gm. of the meal and 600 c.c. of milk upon the afternoon of March 1, and followed this later in the day by the "forced feeding" of 100 gm. of fresh lean beef. This fresh beef was fed by making it into small balls, which were inserted well back in the animal's mouth, after which the jaws and nostrils were held tightly closed

<sup>13</sup> In this process the dog's jaws were separated by means of a piece of wood, six inches long, two and one-half inches wide, and one inch thick, which was provided with a circular hole through the centre. A rubber tube similar to the ordinary stomach tube was then introduced into the stomach of the animal through this opening. After forming a thin soup of the milk and beef meal this mixture was introduced into the tube slowly by means of a small funnel. This method of feeding was found uniformly satisfactory.

and the dog compelled to swallow. Thinking that the ingestion of such a large amount of meat might possibly cause some serious disturbances before morning, the animal was carefully watched until nearly 2 o'clock. Nothing unusual was observed, however, the dog remaining rather inactive during the entire night.

*b. Toxic symptoms.*—At 7.30 A. M. on March 2 the animal became more active than on the previous day, and later in the forenoon muscular twitching and a pronounced parietic condition developed in the posterior limbs. When observed at 3 P. M. the dog was lying quietly in the cage, feeling apparently about the same as earlier in the day. However, when spoken to, she failed to respond as usual, and had apparently lost her sight and hearing. A lighted match held within one-half inch of her opened eye was unnoticed. When placed upon her feet, she showed pronounced symptoms of *ataxia* and walked around in a circle *always to the left*. She did not appear to see the sides of the cage and was continually pushing her nose against the metal lining. Often she would walk entirely around the interior of the cage with her nose constantly against the wall. Frequently, under these circumstances, when reaching a corner she would push hard against the metal as though attempting to force her way out. At such times her neck would be doubled under, and she would apparently use considerable strength in her attempt to force an exit. When taken from the cage and placed upon the floor of the room, she showed symptoms similar to those just mentioned. She walked with a staggering gait in a circle to the left, running her nose with abandon into walls, posts, table legs, glass vessels, and anything which happened to be in the path of her circular movements. She would not respond to a call of any kind and apparently had no power to distinguish sounds. She did not appear to notice the very loud noise produced by bringing two heavy pieces of wood forcibly together close to her ear. The "twitching" movements so very noticeable up to this point now became much less pronounced. At this time the respiration of the animal was much more regular than earlier in the day, being 20; the pulse was 100, and body temperature 38.5° C.

When returned to the cage, she was *constantly in motion*. She could be quieted by smoothing her head or rubbing her ears, but if this was not continued she would suddenly, for no apparent reason, arise and begin moving aimlessly about the cage with droop-

ing head. She would also start up in this impulsive way if the cage was jarred in any manner. After three-quarters of an hour, during which time she had been repeatedly soothed and in which time she had repeatedly gone through the same course of movements, she lay down and remained quiet, with her eyes partly closed. Complete anæsthesia was noted at this juncture, a needle forcibly inserted into her body at various points being absolutely ignored. The customary symptoms persisted until 5.15 P. M., when a change was noted. The dog now became much more active than usual and tumbled violently about the cage. At intervals she would stop her violent movements and fall upon her right side while her legs would be fully extended and stiff. This cataleptic condition soon passed. The dog very often ran out her tongue and acted as if experiencing the presence of a peculiar taste. This process was accompanied by increased salivation, and during it she frequently caught her tongue between her teeth, causing slight laceration.

The cataleptic symptoms passed away at 7.30. and she acted much the same as she did before the symptoms appeared. She had a tendency now to remain for several minutes with her legs folded under her and her nose tightly pressed against the corner of the cage; frequently the pressure was so great as to cause labored breathing. At 8.45 P. M., when the dog was taken from the cage and allowed to walk about the room, instead of going in a circle to the left as before, she walked directly forward. She still suffered from defective sight and hearing, but the ataxic symptoms were less pronounced. That the animal was still under the influence of complete anæsthesia was demonstrated when a needle was run completely through the beast's ear and she made no movement. At 9.30 P. M. urine and feces were passed. When the animal was returned to the cage, she rushed about in the customary violent manner, and after a time sank down, apparently exhausted, and remained very quiet with her eyes closed until nearly midnight.

At 7.30 the next morning the animal was resting, with closed eyes, in a position similar to that in which she had been left at midnight. At this time respiration was 16 and pulse 92. When aroused, she walked with staggering steps for a very short time and then lay down. The tendency to run her nose into the walls of the cage was gone. Recovery from anæsthesia appeared complete, as she reacted very sensitively to a slight prick of the needle.

There was apparently entire recovery of sight and hearing also, and the symptoms of ataxia were absent. When lifted from the cage and placed on the floor of the room, she followed us about much as she did normally, always running in a forward direction, regulating her pace by ours, responding to calls, and carefully avoiding contact with obstacles placed in her path. The return to a normal condition gradually continued, and at 5 P. M. the animal was apparently as normal as at any time since the operation. At 6.30 P. M. she lay down in a comfortable position in the cage and would not respond to a call, but at 7.45 P. M., when she was removed from the cage to the floor of the room, the animal ran about in the usual way and appeared happy. No further change was noted up to 11.30 P. M.

The following day the dog was entirely free from any toxic symptoms, but appeared somewhat stupid. She refused to eat meat, but was forced to take 125 gm. of fresh lean meat administered in small balls according to the method already described. The meat ration was increased to 250 gm. per day and continued for four days longer, during which period there was no recurrence of the toxic symptoms. On the morning of March 8, however, at 9.30 the dog was found lying prone on her right side in the bottom of the cage, making vigorous "running movements" with her anterior and posterior limbs, the former moving the more rapidly. At this time respiration was 39 and pulse 148. When lifted to an upright position, she could not maintain her equilibrium, but immediately sank back upon her side and continued the movements of her limbs. By means of the proper tests the animal was found to be totally blind and deaf and in a condition of absolute anaesthesia. At 10 A. M. the neck of the animal stiffened and the movements of the limbs were superseded by a temporary stiffening of the muscles of the limbs. During this period, if the limbs of the animal were placed in any position other than that assumed by the beast, they would at once return to their original position. These tetanic contractions continued only about ten minutes. Respiration at this point was 48 and pulse 110.

At 11.20 A. M. respiration was slower and deeper, the jaws being tightly closed on lips and tongue, which were slightly lacerated. The "running movements" of all four limbs were still present, although not so pronounced as earlier in the day. The animal continued to lie on her right side, and when rolled over upon her left

side she immediately resumed her former position as soon as she was released. This attempt to cause her to change her position from the right to the left side was made frequently, but always with the same result, *i. e.*, the animal persistently returning to the right-side position. At 12 M. the customary "running movements" of the limbs were replaced by a convulsive movement, which consisted of a forcible drawing of the anterior limbs close to the body in a decidedly cramped position. The posterior limbs were not included in the convulsive movement, but remained entirely inactive.

At 1 P. M. the condition of anaesthesia was absent, as was shown by the resentment exhibited by the animal when her ear was subjected to a slight pinch; respiration was more normal. Sight and hearing had returned at 2.40 P. M., but the anterior limbs were still in a somewhat cramped position. At 3.30 P. M. the animal responded to a call and made an ineffectual attempt to stand, her legs being too weak to support her; at 6 P. M., however, she was more successful in maintaining her equilibrium and succeeded in walking a few steps. Some time later the animal assumed an easy position in the cage and fell into a heavy slumber; she was still in this condition at midnight. The next morning no toxic symptoms could be detected, but the animal was active as usual and appeared happy.

*c. Meat-free diet.* — At this point in the investigation meat was eliminated from the diet, the daily ration being composed of bread and milk. The animal appeared satisfied with this diet, and it was not necessary to employ the "forced feeding" methods previously in use. Although she was given all the food she would eat, the beast lost weight rapidly, and gradually became less active, weak, and somewhat emaciated in appearance. From April 10 to April 16 the weakness increased rapidly, and at 3 P. M. on April 17 the animal was found dead. During the course of the experiment the dog lost about 42 per cent in body weight.

*d. Post-mortem examination.* — The body was very emaciated. 800 c.c. of ascitic fluid was found in the body cavity. Peritoneum was roughened by a fibrinous deposit. Some adhesions with intestines at the site of operation. Upon freeing the adhesions the parietal peritoneum was found covered here and there with villous growths. The omentum was rolled together and contained numerous whitish nodules the size of a small pea. The spleen was very small, but apparently normal. Whitish bodies were scattered throughout the mesentery. The liver was lighter in color than



normal, and its surface was studded with whitish nodules. The membranes of the uterus, tubes, and ovaries were much thickened and contained the same white bodies. The left lung was free, and the right lung was fastened to the dorsal pleura by adhesions. The diaphragmatic pleura and the pleura of the mesocardium contained masses of isolated white nodules. The heart and pericardium seemed normal. The liver was adherent to the intestine at the site of operation by adhesions which were easily broken down. The Spigelian lobe was entirely covered with a mass of omentum filled with white nodules. The pancreas was transformed into a cirrhotic mass of tissue. The fistula wound seemed perfectly satisfactory. The fistula was about 2 cm. in length. There were no anastomoses.

Smears from the whitish nodules of the liver showed the presence of numerous tubercle bacilli.

2. **Experiment II.** *a. Operation and preliminary feeding.* — The subject of this experiment (Dog No. 4) was a bitch weighing a little over 13 kg. The Eck fistula was established on the evening of April 15. This animal was fed meat from the start of the feeding experiment. The dog evinced no distaste for the diet, as was so strikingly portrayed in Experiment I by Dog No. 2. After meat had been fed for four days we began to look for some indication of the toxic signs observed in Experiment I. However the typical symptoms did not appear even after the animal had partaken of the meat diet for a month. At this point we injected sodium carbamate<sup>14</sup> (0.3 gm. per kilogram body weight) into the jugular vein, in an attempt to verify the findings of Pawlow and his associates in this connection. To our surprise the injection was not followed by any toxic symptoms such as Pawlow had observed. In our experiment there was no immediate effect except an acceleration of respiration and circulation. Later the dog became somewhat stupid, and for three days subsequently the urine was alkaline in reaction.

*b. Feeding of Liebig's extract.* — On the following day the feeding of fresh lean beef was again begun, and continued for twenty-nine days longer. The meat ration had now been fed through a period of two months, and no evidences of the toxic disturbances

<sup>14</sup> This sodium carbamate was made for the author at the Harrison Chemical Laboratory of the University of Pennsylvania through the courtesy of Professor E. F. Smith.



observed in Experiment I had been in evidence. Thinking that perhaps the extractives of the meat rather than the protein constituents might have been responsible for the toxic symptoms previously observed and that possibly this animal was much more resistant to the influence of the extractives, therefore necessitating the ingestion of a much greater quantity of the fresh meat to obtain the same result, we concluded to feed the extractives in a concentrated form. To this end 20-50 gm. of Liebig's extract was daily added to the diet of fresh lean beef. Nothing out of the ordinary was observed in connection with the animal until the extract had been eaten for six days; at this time a weakness was observed in the posterior limbs. This paretic condition gradually increased, and on the evening of June 24 it was very pronounced. No albuminuria was observed after feeding Liebig's extract, as was reported by Macleod.<sup>15</sup>

*c. Toxic symptoms.* — On the morning of June 25, after Liebig's extract had been added to the diet of fresh lean beef for a period of ten days, a series of toxic symptoms similar to those observed in Experiment I was observed. At 9 A. M. the dog was found staggering about the cage, showing signs of great weakness in both the anterior and posterior limbs. At this time the animal's respiration was 120 and the pulse 102. The beast was blind and deaf, and exhibited pronounced ataxic symptoms. At 10 A. M. the dog was in a pronounced paretic condition, being absolutely powerless to use either her anterior or posterior limbs. Respiration at this time was 120 and pulse 112. Anæsthesia was not complete in this case, there being slight reactions to needle pricks in the region of the back and sides of the trunk.

At 11.30 A. M. the animal started up suddenly with no evident purpose and for no apparent reason. She staggered about the room, running into posts, walls, and chairs, and finally sank down apparently completely exhausted. In a short time she arose and staggered about in a zigzag manner. Coming in contact with a short stairway, she made persistent ineffectual efforts to ascend, and finally succeeded in mounting a series of four steps. Soon after this the animal lay down upon the floor and exhibited the stiffening of the limbs observed with Dog No. 2. The knee jerk was exaggerated, and clonic contractions of the limbs were in evidence. At 12.15 P. M. respiration was 140 and pulse 128.

<sup>15</sup> MACLEOD: *Loc. cit.*

The dog was now returned to the cage, and at 1 P. M. was fed 50 gm. of Liebig's extract. The animal remained lying prone upon her right side, with limbs extended and pliable but inactive, for over four hours. At 5.30 P. M. a muscular twitching was noted in the anterior limbs, and almost immediately both the anterior and the posterior limbs became stiff. Simultaneously the neck became stiff and rigid, sufficiently so to allow the body of the animal to be lifted from the floor by placing the hand under her head. This condition was not typical opisthotonus. Anaesthesia was complete, as was evidenced by the failure of the animal to react to various stimuli. Proper tests also indicated the absence of sight and hearing. At 7.30 P. M. the animal died.

During the course of the seventy days of the experiment the animal had lost about 15 per cent in body weight. The percentage loss in weight was only about one-third as great in the case of this dog as was noted in the case of Dog No. 2, and further the conditions of this experiment differ from those of Experiment I in that, in this instance, the subject retained her appetite throughout the course of the experiment and ate the meat eagerly at all times, whereas in the first experiment a dainty appetite and an antipathy to meat were indicated from the inception of the experiment.

*d. Post-mortem examination.*—The body of the dog was not emaciated, as was the case in the instance of Dog No. 2. Organs were normal. Adhesions at the site of fistula. Fistula opening about 2 cm. long, and edge of the opening covered with endothelium. Gall bladder greatly distended. Upon injecting the body no injection mass passed to the liver. No anastomoses were indicated, and the absence of tubercular nodules was apparent.

**3. Experiment III.** *a. Operation and preliminary feeding.*—The subject of this experiment was a bitch weighing about 11 kg. Eck's operation was performed, and on May 14 the dog was placed upon a daily ration of 1 pound of fresh lean meat. The animal ate the proffered meat with evident relish throughout the twenty days she was maintained upon this ration. During this time the animal appeared in all respects as a normal dog. At this point sodium carbamate was injected.

*b. Injection of sodium carbamate and feeding of Liebig's extract and sodium carbamate.*—Upon the morning of June 3, the twenty-first day of the experiment, sodium carbamate (0.6 gm. per kilo-

gram of body weight) was injected into the right jugular vein of the animal. This amount of carbamate, although far in excess of that injected by Pawlow and his associates, was followed by none of the toxic symptoms observed by this eminent investigator. The only effect of the injection was a slightly increased respiration and circulation. This acceleration continued but a short time and was followed by a period during which the beast remained very quiet and inactive. All her faculties were intact, but she refused to respond to a call and stood quietly with drooping head and was somewhat stuporous. The urine for the next two days was alkaline in reaction.

The beef diet was now continued for sixteen days longer and was again unaccompanied by any abnormal symptoms. At this point Liebig's extract was added to the diet during a period of three days, and nothing noteworthy developed from its inclusion in the dietary. On June 23 sodium carbonate was introduced into the dog's stomach by means of a tube, to neutralize the acidity of the gastric juice, after which sodium carbamate (0.6 gm. per kilogram body weight) was fed by the same means. A large part of the carbamate was vomited by the animal, but was immediately reintroduced into her stomach. The feeding of this carbamate was *absolutely without effect*, contrary to the findings of Pawlow and his associates. Two days later, on June 25, sodium carbamate was again fed in equivalent amount in *dry* form and was all retained. The result of the feeding was again negative, the animal exhibiting *no toxic symptoms* of any form. The customary beef diet was now fed for two days longer, at which point the animal was placed on a diet of bread, milk, and Liebig's extract.

*c. Meat-free diet plus Liebig's extract.*— It was shown in Experiment II that the daily addition of Liebig's extract to the fresh meat ration of an animal for a period of ten days was followed by a series of toxic symptoms which culminated in a few hours in the death of the animal, notwithstanding the fact that previous to the feeding of the Liebig's extract the animal had been maintained for a period of two months upon a daily ration of fresh lean beef, and further that on the thirty-first day of the experiment sodium carbamate had been injected intravenously. In the experiment under consideration (III) the conditions were similar to those just recited in that the animal had been maintained upon a ration of fresh lean beef for a long period and in addition had received

sodium carbamate, both by intravenous injection and by the mouth, and all without the slightest indication of any toxic disturbance.

The result obtained in Experiment II indicated that, in the case of this animal, Liebig's extract was necessary to the production of the toxic symptoms already mentioned. There was, however, no proof that Liebig's extract could bring about the disturbances on a daily ration from which the meat was eliminated. We now proceeded to test this point, and on June 28 placed the dog on a daily ration of one quart of milk, 60 gm. of bread, and 60 gm. of Liebig's extract. This diet was continued for a period of thirty-one days, and instead of the diet creating anything of an undesirable nature the dog was apparently very happy and contented, and during the time this diet was fed gained steadily in body weight, the aggregate gain for the period of thirty-one days being 9.1 per cent.

*d. Meat diet plus Liebig's extract.*—Following the failure of the addition of Liebig's extract to a diet of milk and bread to cause any toxic symptoms, a return was made to the ordinary meat diet. During the following fifty-three days the animal was fed daily 1 pound of fresh lean beef. The only result of this beef diet was a gradual loss in weight, the animal appearing unchanged in all other particulars. The dog had now been under investigation for one hundred and twenty-eight days, and had been fed a diet of fresh lean meat for ninety-seven days, and a diet of milk, bread, and Liebig's extract for thirty-one days, and still no disturbances of a toxic character were in evidence. At this juncture it was determined to add Liebig's extract to the meat diet the animal was then receiving, and, beginning September 20, the daily ration was modified to include 1 pound of fresh lean meat and 50 gm. of Liebig's extract.

Upon this diet the dog soon began to exhibit definite symptoms of increasing weakness. On the afternoon of September 26 the animal became suddenly much weaker than usual, and when observed at 2.30 P. M. she was staggering slowly and unsteadily about the cage, but avoided coming in contact with the walls of the cage, and her sense of hearing was apparently normal. At this time her respiration was 188 and pulse 164. At 3.00 P. M. her condition suddenly changed, and she began to walk rapidly about the interior of the cage to the left. When lifted from the cage to the floor of the room, the dog walked for the most part straight ahead with

a staggering gait, but made an occasional wide circle to the left. At 5.06 P. M. the respiration had become very rapid and labored, registering 224, while the pulse was 186 and the rectal temperature  $42^{\circ}$  C. The dog was now lying on her right side and made very rapid "running movements" with both the anterior and the posterior limbs. At 5.45 P. M. the animal's limbs had begun to stiffen perceptibly, although the movements continued. At this time about 200 c.c. of a yellowish-colored fluid was vomited, and immediately after 20 c.c. of urine was voided spontaneously. For a short period at this juncture the respiration, the pulse, and the movements of the legs were less rapid, but soon returned to the former condition. When supported, the animal made her feet go rapidly along the floor, overlapping to the left each time. These symptoms were followed by complete blindness, deafness, and anaesthesia at 6.00 P. M. At 6.30 P. M. the animal ceased breathing. The dog lost 14 per cent body weight during the course of the one hundred and thirty-five days of the experiment.

*c. Post-mortem examination.* — The body was injected, and none of the injection mass passed to the liver. Gall bladder greatly distended and tightly adherent to the liver. Pancreas normal. Spleen normal except for a dark area along the outer edge. Liver and other organs free from tubercular nodules noted at the autopsy on Dog No. 2. Intestines normal. Complicated adhesions at the site of the Eck fistula, and upon dissecting out the fistula an opening of  $1\frac{1}{2}$  cm. was revealed. Four and one-half months having elapsed since the operation was performed, it is evident that the fistula opening was originally no doubt much larger than this. Even at this time, however, there was undoubtedly a free opening between the portal vein and the vena cava. There were no anastomoses.

4. **Experiment IV.** *a. Operation and preliminary feeding.* — A bitch weighing about 11 kg. was used as the subject of this experiment (Dog No. 1), the fistula being established on February 8. For a few days at the beginning of the experiment the animal was fed a diet rich in carbohydrate material, with the idea that sugar would appear in the urine provided the fistula had been successfully established. However sugar could not be detected in the urine, and apparently there was no trace of glycosuria. Since these experiments were completed, Filippi<sup>16</sup> has also observed that glyco-

<sup>16</sup> FILIPPI: *Zeitschrift für Biologie*, 1907, xlix, pp. 511-557.

suria does not follow the feeding of starch to Eck-fistula dogs. This investigator claims, however, that such animals have a lowered tolerance for sugars given in solution or for lactose in milk, a smaller amount leading to glycosuria than in a normal animal.

*b. Meat diet.*—From February 16 until the end of the experiment the dog was fed a meat diet. At first 300 gm. of fresh lean beef was fed, but soon the appetite diminished, and the amount of beef fed daily was reduced to 200 gm. and finally to 150 gm. At the end of the experiment it was necessary to use our "forced feeding" methods, as the dog could not otherwise be induced to partake of the meat.

The subject of this experiment was an extremely restless, uneasy, and irritable individual. She had been confined in the cage only a few days when she began to chew a hole in the heavy-wire partition of the cage and to tear out large pieces from the wooden portion of her house. This extreme irritability was not noted to such an extent after February 17. When the plaster cast was removed from the wound and replaced by a bandage, it was noted that the wound had not granulated very extensively and that the edges were one-half inch apart in some places. The tendency for the wounds to open in this manner was noted to a much more extensive degree in the experiments of Pawlow and his associates.<sup>17</sup> The dog gradually lost in body weight and appetite, and became decidedly weaker. On March 7 at 9 A. M. she vomited about 100 c.c. of a yellow frothy fluid and passed a fairly large liquid stool. Subsequently she appeared extremely weakened and became stupid. The animal continued to get progressively weaker, and at 10.45 ceased breathing without having given evidence of the toxic symptoms observed in the experiments already discussed. The dog lost 20 per cent in body weight during the course of the experiment.

*c. Post-mortem examination.*—The liver was normal in color and contained no tubercular nodules. The gall bladder was fully distended with bile. All the organs of the abdominal cavity appeared normal, except that the intestines contained blood-stained feces. There was no evident explanation for this. No sign of peritonitis. Organs of the thorax were normal. No adhesions between the intestines and the site of operation.

Upon dissection of the dog after injection of the portal system

<sup>17</sup> HAHN, MASSEN, NENCKI and PAWLOW: *Loc. cit.*



it was found that the portal vein above the ligature, which was intact, contained some injection mass. Dissection of the parts revealed a small branch of the portal emptying into another branch of the portal just before it passed into the liver, about 2 inches above the ligature. This branch was traced back to the venous plexus of the smaller curvature of the stomach, which plexus anastomosed with a fairly large branch of the splenic vein. Section of the liver showed that some of the large vessels were filled with injection mass, but it would seem that this occurred as a backward flow from the vena cava, which was not tied off before injection. The fistula opening was rather small, about 1 cm. long. The edges of the opening were covered with endothelium, and the stitches were healed into the tissues. It is probable that the opening was rather small at the time of operation, and that it subsequently closed further, and that pressure in the portal system caused the anastomoses to open. This dog is of interest in connection with Queriolo and Massini's<sup>18</sup> work, as their dogs probably had similar anastomoses.

**5. Experiment V. a. Operation and feeding tests.**—The subject of this experiment (Dog No. 5) was a bitch weighing about 5 kg. The fistula was established May 4. A ration consisting of one-half pound of fresh lean beef was fed daily. The dog gradually lost body weight, but gave no indication of anything abnormal until about the middle of June, when it was observed that she was growing perceptibly weaker. By June 18 she was extremely weak and walked with a staggering gait, although she wagged her tail and appeared happy when caressed. The posterior limbs were the weaker. On June 21 50 gm. of Liebig's extract was fed and was at once vomited. On June 22 the paretic condition of the posterior limbs was more pronounced. On this day at 10 A. M. she was fed sodium carbamate (0.6 gm. per kilogram body weight) with negative effect. The next day the dog possessed very little power over her hind limbs. When lifted from the cage to the floor, she was unable to make any use of her hind limbs, but sprawled about in "frog fashion." However, when her head was stroked, she wagged her tail as before and seemed more or less contented. At 10.50 A. M. on this day she was fed sodium carbamate (1.2 gm.

<sup>18</sup> QUERIOLO and MASSINI: MOLESCHOTT'S *Untersuchungen zur Naturlehre der Menschen und der Thiere*, 1895, xv, 226.



per kilogram body weight), and again the only effect was a quickened respiration and pulse. At 11 A. M. the animal passed a soft stool; at 11.20 she passed a colorless fluid from the rectum, which was strongly alkaline in reaction, and at 11.40 a greenish fluid was passed which was less alkaline than the colorless fluid.

The following day the animal exhibited evidences of a parietic condition. Sight and hearing were somewhat affected, and the sense of feeling was blunted to a certain degree, but there were no decided toxic symptoms such as have already been cited as occurring in some of the previous experiments. On June 26 the animal ate practically nothing and died about noon. She lost 24 per cent in body weight during the course of the experiment.

*b. Post-mortem examination.*—The dog was injected, and no injection mass passed to the liver. Gall bladder was distended. Slight adhesions at the site of operation. Fistula opening about  $1\frac{1}{2}$  cm. long and no anastomoses. No tubercular nodules could be detected.

**6. Experiment VI.** *a. Operation and preliminary feeding.*—The subject of this experiment was a bitch (Dog No. 3) weighing about 6 kg. She was operated on early in March, and from March 9 to March 21 inclusive she received a diet of milk, bread, and potatoes. There were no traces of sugar in the urine during this period.

*b. Meat diet.*—From June 22 until the termination of the experiment the customary diet of fresh lean beef was fed. During the whole experiment the animal acted like a normal dog in every particular; she had a good appetite and did not lose body weight, as the other animals had done.

*c. Exploratory operation.*—Inasmuch as the animal appeared to be in such a normal condition, we considered it probable that the fistula was not properly established, or that the effect of the fistula if properly established had probably been nullified through the establishment of a collateral circulation. To determine the truth regarding the matter, it was deemed advisable to place the dog under the influence of an anæsthetic and open the abdomen and make a careful examination of the fistula. This was done on April 23. At this time we could find no evidence of any veins opening above the ligature of the portal. The liver was unusually lobulated in the lower portion on the right side, and some of the small lobes were firmly adherent to the kidney and the intestinal

peritoneum; these adhesions were broken down. The lowest of the lobules which was especially adherent was removed (10 gm.). The omentum was then drawn down to the posterior peritoneal surface by a suture, the purpose being to prevent the formation of adhesions between the liver and the intestines. There were no adhesions among the intestines, and none to the peritoneal wall.

*d. Meat diet.*— Since the conditions were found satisfactory when the abdomen was opened on June 23, the meat diet was continued as soon as the dog recovered from the effects of the exploratory operation. No toxic symptoms resulted from the feeding of the meat, however, and as the cage was needed for another dog which had just been operated upon (Dog No. 5), the animal under experimentation was killed on May 11. The idea of feeding Liebig's extract did not suggest itself to us until later (June 15), otherwise a test similar to those described in Experiments II and III would have been made.

*e. Post-mortem examination.*— All organs appeared normal. The fistula was satisfactorily established, the opening being  $1\frac{1}{2}$  cm. long. After the animal was injected, no injection mass was found in the liver. There were no anastomoses.

#### IV. CONTROL EXPERIMENTS ON NORMAL DOGS.

1. *Injection of sodium carbamate: Dog No. 20.*— This dog, weighing 6 kg., was maintained on a meat diet for twenty-one days and then injected with 0.3 gm., 0.6 gm., and 1.2 gm. of sodium carbamate per kilogram body weight, an interval of two days elapsing between successive injections. The carbamate was dissolved in physiological salt solution, a 5 per cent carbamate solution being employed. The toxic symptoms reported by Pawlow and his associates were not observed in any instance. No influence was noted beyond an acceleration of the respiration and the pulse and a slight diuresis. The urine was alkaline in reaction for two days following.

*Dog No. 21.*— A dog, weighing 9.8 kg., was maintained on a diet of milk and bread and injected similarly to Dog No. 20. The carbamate had no apparent influence.

*Dog No. 22.*— A dog, weighing 6.6 kg., was maintained on a meat diet for eighteen days and then injected twice with 0.6 gm. and once with 2.4 gm. of sodium carbamate per kilogram body weight. There was no appreciable effect.

2. *Feeding of sodium carbamate: Dog No. 21.*—A dog, weighing about 9 kg., was fed a meat diet for four days. The animal had previously been used for an injection test. The acidity of the gastric juice was neutralized by sodium carbonate, and sodium carbamate (1.2 gm. per kilogram body weight) was then fed in powder form. No toxic effect was observed, in this point agreeing with the findings of Pawlow and his associates.

*Dog No. 23.*—A subject was a bitch weighing 7.3 kg. She was fed a meat diet for six days, at the end of which period the acidity of the gastric juice was neutralized by sodium carbonate, the neutralization being followed by the feeding of sodium carbamate (2.4 gm. per kilogram body weight) in dry form. No toxic effect was evident.

3. *Injection of Liebig's extract: Dog No. 21.*—This animal, weighing about 9 kg., had served as the subject of a sodium carbamate feeding experiment. Following this, she was placed on a meat diet for twenty-six days, at the end of which time Liebig's extract was injected into the right jugular vein. For the injection 30 gm. of the extract was dissolved in salt solution of such a strength that the resulting mixture was approximately isotonic. Twenty cubic centimetres of this solution was injected slowly, sixteen minutes being consumed in the process. The dog was very restless during the injection of the 20 c.c., and at the termination of the injection both respiration and heart stopped, and although artificial respiration served to produce an apparent recovery, yet the animal died about one hour later. The final cessation of respiration and of heart action was preceded by a few pronounced convulsive movements.

*Dog No. 23.*—This dog had previously been the subject of a sodium carbamate feeding experiment. It was apparent, from the result of the injection of Liebig's extract in the above experiment (Dog No. 21), that the extract had been injected too rapidly and in too large an amount. In the present experiment it was determined to inject the extract more slowly and in smaller amount. The animal, which had been fed a meat diet for twenty-four days, weighed about 7 kg. At the injection of 1 c.c. on January 26 an accelerated respiration and pulse were noted, accompanied by a pronounced salivation; 2 c.c. slowed both respiration and pulse, salivation remaining unchanged; after the injection of 3 c.c. the pulse was barely perceptible. At this point pronounced convulsive move-

ments were observed, accompanied by the spontaneous passage of urine. On the next day the injection was continued, 3.4 c.c. being injected in one minute, causing the respiration to increase from 20 to 30 and the pulse from 84 to 160. There was profuse salivation. During the next thirty minutes 32.8 c.c. of the extract was injected, the respiration at that time being 28 and the pulse 80. A small amount of feces and about 10 c.c. of very dark-colored urine was now passed. During the next twelve minutes 10 c.c. of extract was injected. Five minutes later about 300 c.c. of a thick mucous material, containing a little bile, was vomited. Respiration was now 36 and pulse 70. In a space of fifteen minutes 10.8 c.c. of the extract was next injected, increasing the rate of respiration to 48 and leaving the pulse unchanged (70). The last injection was one of 5 c.c. made during a five-minute period. This was instrumental in raising the respiratory rate to 56, while the pulse remained at 70. One-half hour after this last injection respiration was 48 and pulse 140. The dog remained very quiet, but there was no sign of any such toxic symptoms as were observed after feeding the extract to Eck-fistula dogs.

The third and final injection of the extract occurred on January 30. Before the injection respiration was 20, pulse 96, and body temperature  $38.5^{\circ}$  C. Six and one-half cubic centimetres was injected in three minutes, the injection being followed by a depression of both respiration and pulse, the former being 12 and the latter 68; body temperature was now  $38.8^{\circ}$  C. In the next ten minutes 15 c.c. of the extract was injected; respiration now 12, pulse 132, and body temperature  $40.5^{\circ}$  C. Urine and feces were now passed. In the next seventeen minutes 20 c.c. was injected, causing a rise in body temperature to  $42.5^{\circ}$  C., both respiration and pulse remaining unchanged. More urine and feces were passed, the urine being extremely dark, almost as much so as the original solution of Liebig's extract. The experiment closed eight minutes later, after an injection of 10 c.c. of extract. This was followed by very violent retching movements which culminated in stercoraceous vomiting. Respiration ceased and artificial respiration was used without effect. Body temperature was now  $44.5^{\circ}$  C. During the course of this third series of injections no such acceleration of respiration was observed as occurred during the second series. On the other hand, the body temperature of the animal increased very remarkably during the third series of injections, whereas there

was but slight variation from the normal during the course of the injections previously made.

4. *Feeding of Liebig's extract: Dog No. 20.* — This subject was a dog weighing 6.0 kg. which had previously been used in an injection experiment. The animal was fed a meat diet for twenty-eight days, at the end of which time Liebig's extract was added to the diet for a period of eighteen days. No toxic symptoms were observed, such as those which followed the feeding of this extract to dogs after the establishment of the Eck fistula.

*Dog No. 22.* — This dog had previously been used in an injection experiment and weighed about 6 kg. The animal was maintained on a meat diet for ten days, after which time Liebig's extract was added to the daily ration for a period of twenty-one days. No deviation from the normal was noted.

#### V. CONCLUSIONS.

1. The establishment of the Eck fistula in dogs may be followed by the appearance of pronounced toxic symptoms when the animals thus operated upon are fed a diet containing a large amount of meat. However, in our experiments, such a diet did not always produce this result, even when the post-mortem examination showed the portal vein to be tightly ligatured, an ample fistulous opening between this vein and the vena cava, and no signs of a collateral circulation.

2. Eck-fistula dogs which failed to show any toxic symptoms when fed a meat diet for a long period invariably exhibited these toxic signs after Liebig's extract had been added to the meat diet for a few days.

3. The toxic symptoms noted in Eck-fistula animals, after feeding a meat diet or such a diet supplemented by Liebig's extract, may include ataxia, tetanus, catalepsy, and paresis as well as complete anæsthesia and total loss of sight and hearing. These symptoms ordinarily terminate fatally, but under certain conditions the elimination of the disturbing factors from the diet may be followed by the recovery of the animal.

4. No toxic symptoms followed the feeding of a *meat-free* diet to Eck-fistula dogs, even when such diet was supplemented by the addition of Liebig's extract.

5. The feeding of sodium carbamate to Eck-fistula dogs, as well

as the intravenous injection of such animals with this salt, was productive of no such toxic symptoms as those observed after meat and extract feeding.

6. The feeding of sodium carbamate or Liebig's extract to normal dogs, maintained upon a meat ration, as well as the intravenous injection of either of these substances, failed to produce such toxic symptoms as were observed in Eck-fistula dogs which were fed upon a diet of meat or upon a meat ration supplemented by the addition of Liebig's extract.

7. Glycosuria did not follow the feeding of carbohydrate food to Eck-fistula animals.

8. There were no signs of albuminuria in Eck-fistula animals after the feeding of Liebig's extract.

9. The establishment of Eck's fistula was sometimes followed by a period in which the animal was extremely nervous, restless, and irritable.

10. The Eck-fistula dogs invariably lost weight, the loss in weight for the different animals ranging from 14 per cent to 42 per cent. The dog which lost 42 per cent of its body weight was shown by post-mortem examination to be tubercular.

11. Meat was refused by Eck-fistula animals after recovery from the toxic symptoms induced by meat feeding.

12. Inasmuch as the feeding of Liebig's extract for periods of seven and ten days in connection with a diet of fresh lean beef was followed by toxic symptoms (Experiments II and III) which quickly resulted fatally, and, further, whereas no such toxic effect was apparent from the feeding of this extract for a period of thirty-one days in connection with a diet containing no meat, the indications are that one or more of the constituents of the Liebig's extract was responsible for the toxic symptoms observed, but that such constituents are toxic, according to our observations, only in the presence of a meat diet, and that when fed in connection with a diet from which the meat is eliminated they are inert.





PROCEEDINGS OF THE AMERICAN PHYSIO-  
LOGICAL SOCIETY.

TWENTIETH ANNUAL MEETING.

CHICAGO, DECEMBER 31, 1907, and JANUARY 1 and 2, 1908.



PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL  
SOCIETY.

THE ANTAGONISTIC ACTION OF CALCIUM UPON  
THE INHIBITORY EFFECT OF MAGNESIUM. —  
A DEMONSTRATION.

BY S. J. MELTZER AND JOHN AUER.

CALCIUM and magnesium are closely related chemically and are constant companions in the tissues of the animal body. It is assumed that both elements exert similar biological actions and that both cause or favor inhibition. The facts which lead up to this now prevailing view are well known and will not be discussed here. The writers have recently established by various experiments that calcium is to a great extent strikingly antagonistic to the inhibitory effect of magnesium. The demonstration consists in showing how the paralysis and anæsthesia of an animal brought on by magnesium salt can be made to disappear in a minute's time by the injection of a calcium salt. Similar relations exist in plant biology and "therapeutics." When growth is unfavorable on account of the presence of too much magnesium in the soil, the cure for it is "liming." The companionship of the two elements in the animal tissues means, therefore, not an aggravation, but, partly at least, a neutralization of effects, or, to use a happy expression of Loeb, it means the production of physiologically balanced conditions.

THE CILIAM STUDIED COMPARATIVELY AS A KEY TO  
THE STRUCTURE OF CONTRACTILE PROTOPLASM.

BY O. P. DELLINGER (by invitation).

CILIA are extensions of relatively pure contractile tissue outside the cell. Reagents that preserve cilia should preserve similar structural

elements in the cell. Of the twenty-eight reagents tested osmic acid only preserved cilia as they appear in life. All other reagents destroyed the identity of the cilia more or less. Almost any structure from granules to alveoli may be obtained if the appropriate killing reagent is used. Killing agents probably more often alter or destroy existing structures than produce "artifact" structures.

The prevailing opinion is that flagella are homogeneous. The flagellum of *Euglena* is composed of four fibres arranged spirally. Specimens were teased fresh and photographed. Other tissues showing a fibrillar structure are: cilia of *Stylonychia*, the flagellum of *Chilomonas*, axil filament of pseudopods of *Actinosphaerium*, myonemes of *Vorticella* and *Stentor*, the ectosarc and endosarc of *Amoeba*, and smooth muscle.

Alveoli are often found collected in the angles of a reticulum. Probably a fibrillar reticulum is always present in protoplasm that shows an alveolar structure.

#### FURTHER OBSERVATIONS ON THE PARENTERAL UTILIZATION OF CARBOHYDRATES.

By LAFAYETTE B. MENDEL.

In an earlier communication it was shown that the parenteral introduction of soluble carbohydrates for which the animal organism possesses no specific digestive enzymes is followed by a speedy elimination of the injected substance in considerable quantity. Further observations on the behavior of sucrose indicate that the sugar may reappear in the urine in amounts reaching over 90 per cent of that introduced by subcutaneous or intraperitoneal injection in dogs, cats, and rabbits. As yet we have no evidence that the sugar retained is utilized by excretion into the alimentary tract and reabsorption after inversion. An occasional feature of such experiments is the elimination of levorotatory substances, probably conjugated glycuronates. Attempts to induce an adaptation and better utilization of the sugar by repeated parenteral injections have been unsuccessful; neither is there a better utilization in starving animals.

Soluble starch introduced parenterally in rabbits is recovered only in part in the urine, in the form of dextrin-like compounds. The utilization (or retention) is most extensive after subcutaneous in-

jection, less after intraperitoneal, and least after intravenous introduction. Tissue amylases apparently play some rôle in this. A complete utilization, such as Moscatti has claimed for starch suspension, was never observed.

#### PROTEIN METABOLISM IN THE DOG—NO. 2. THE INFLUENCE OF LOW CALORIC VALUES OF NITROGEN ON METABOLISM.

BY EMIL OSTERBERG AND C. G. L. WOLF.

ANIMALS were carried through a period of eight days of starvation, with subsequent four-day periods of twenty, thirty, forty, thirty, and twenty calories of protein feeding with blood albumin. The nitrogen and sulphur constituents, phosphorus and chlorin of the urine, and the nitrogen, sulphur, and phosphorus of the feces were determined. The results show that after inanition the retention of sulphur is relatively very much greater than that of nitrogen. The elimination of ammonia follows pretty well the trend of the nitrogen elimination during periods of protein feeding, but is relatively lower than during starvation or when adequate amounts of food containing no nitrogen are administered. During protein feeding the creatinin remains practically constant, with varying amounts of nitrogen ingested. The creatin produced by starvation is inhibited by very small amounts of protein feeding. The undetermined nitrogen follows to a certain extent the amount of protein ingested, but the nitrogen curve rises less rapidly; relatively less undetermined nitrogen is excreted with high protein diet. This is not altogether the case for neutral sulphur. The ethereal sulphur is fairly constant throughout the experiment, and the amount of ethereal sulphur bears no relation to the amount of indican excreted.

#### THE REACTION OF AMOEBA TO STIMULI OF SMALL AREA.

BY J. F. MCLENDON (by invitation).

AMOEBA proteus does not respond to mechanical stimuli of small area unless the stimulation is repeated at intervals of time not ex-

ceeding one to three seconds. Even when a glass needle was stuck through the Amœba no response was observed. When stimulated chemically by pricking with a copper needle of extreme fineness, the stimulus was conducted through the protoplasm with a speed not less than and probably greater than the movement of the hydrogen ion in aqueous solution.

In the food taking of the amœba, the advancing edge is stimulated at the point of contact with the food and contracts. The advance of portions on each side of this point continues until the food is surrounded. Many factors complicate the process, but these factors probably enter in in most cases.

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#### THE EFFECTS OF PROLONGED CENTRIFUGAL FORCE ON PARAMŒCIUM.

By J. F. McCLENDON (by invitation).

THE nuclei of Paramœcium (especially the chromatin portions) are heavier than the endosarc and are precipitated to one end of the organism by the centrifuge. Paramœcium caudatum was kept alive in the centrifuge from five minutes to seven days. After removal the nuclei slowly regained their normal positions, but in some cases this process took several generations of the organism for completion. The rate of division is greater in Paramœcia that have been centrifuged than in the control. The experiments are being continued to determine what other changes take place.

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#### THE EFFECT OF STIMULATION OF THE VAGI UPON THE ONSET AND DEVELOPMENT OF RIGOR MORTIS OF THE MAMMALIAN HEART.

By DON R. JOSEPH AND S. J. MELTZER.

STIMULATION of motor nerves hastens the onset of rigor mortis of the corresponding skeletal muscles. On the hypothesis that stimulation of inhibitory nerves might retard the development of rigor the effect of stimulation of the vagi upon the onset of cardiac rigor was studied. The results did not bear out the hypothesis, and the reverse was found to take place. The observations were made on

42 dogs, 16 cats, and 10 rabbits. Death was caused by bleeding and wide opening of the thorax. There was practically in all animals a distinct difference between the hearts of animals whose vagi were stimulated and those of the controls. The hearts of the stimulated animals stopped beating earlier, the rigor set in sooner and attained its maximum in a shorter time, than in the controls. It was found, however, that the rigor of the skeletal muscles also constantly appeared earlier in the animals whose vagi were stimulated. The most plausible explanation would seem to be that a certain degree of anemia resulting from the frequent stoppages or retardations of the heart beats might be the essential cause of the earlier onset of the rigor of the heart as well as of the skeletal muscles.

#### ON DIFFERENCES BETWEEN THE BULBAR AND SPINAL VASOMOTOR CELLS.

BY W. T. PORTER AND W. I. CLARK.

THE vasomotor nervous system is composed of bulbar, spinal, and sympathetic neurons. Were these all of one order, they would react equally to the same stimulus. In other words, the sciatic reflex and the depressor reflex should both be increased or both be diminished by the action of the same agent. The authors find that the several neurons are affected in different ways by the same drug. Curare, for example, affects the depressor reflex in one way, and the sciatic reflex in another. The experiments seem to demonstrate a specific difference between the bulbar and the spinal motor cells. It is hoped that a therapy of the vasomotor cells may later be established.

#### A COMPARATIVE STUDY OF VASOMOTOR REFLEXES.

BY W. T. PORTER AND R. RICHARDSON.

INDUCTION currents of the same intensity were applied to the sciatic and the brachial nerves of the curarized rabbit, cat, dog, guinea-pig, rat, and hen, and the consequent rise in blood pressure measured. The level of the blood pressure at the beginning of stimulation was taken into account by expressing the change in blood pressure as a percentage of this level. The percentile changes in each animal were as follows:



	Sciatic.	Brachial.
Rabbit . . . . .	56	47
Cat . . . . .	47	45
Dog . . . . .	25	30
Guinea-pig . . . . .	34	33
Rat . . . . .	55	41
Hen . . . . .	54	41

These figures may be changed slightly by the addition of new material. They permit the conclusion that, on the whole, the vasomotor relations in these animals are fundamentally alike.

#### THE REACTIONS OF PERIPHERAL VASOMOTOR AREAS.

BY W. T. PORTER AND E. H. PRATT.

AN artificial circulation is established through the hind limb of a cat, and the flow measured by a counter which records the drops flowing out of the femoral vein. All connection between the blood vessels in the limb and those in the body is shut off. If the carotid artery be now opened, the general blood pressure will fall sharply. The vessels in the isolated limb will then constrict. If the general blood pressure be sharply raised, the vessels in the isolated limb will dilate. Thus variations in the general blood pressure give rise to a protective reflex, tending to raise the blood pressure when it has fallen, and to lower it when too high. Similar results are gained when the limb is placed in a plethysmograph.

#### SURVIVAL OF TISSUES AND ORGANS UNDER PERFUSION.

BY C. C. GUTHRIE.

It has been shown<sup>1</sup> that in the excised mammalian heart which has not been previously asphyxiated, changes of pressure in the coronary vessels cause little or no change in the rate of beat. The heart that has been asphyxiated by stopping the circulation for a time, responds

<sup>1</sup> GUTHRIE and PIKE: *This journal*, 1907, xviii, p. 14.

readily to the changes in pressure. Increase in pressure augments the rate, and decrease in pressure diminishes the rate.

This would seem to indicate the presence of a local controlling mechanism, relatively susceptible to anæmia.

The nature of this mechanism is being further investigated by studying the behavior of unasphyxiated and asphyxiated hearts under the influence of drugs. From the results thus far obtained it would seem that certain differences exist.

#### A STUDY OF THE RESPIRATORY AND CARDIAC ACTIVITIES AND THE BLOOD PRESSURE IN THE SKATE AFTER INTRAVENOUS INJECTIONS OF SALT SOLUTIONS.

By IDA H. HYDE.

SOLUTIONS of sodium chloride, potassium chloride, calcium chloride, magnesium sulphate, magnesium chloride, sodium carbonate, urea, sodium hydrate, hydrochloric acid, barium chloride, sodium sulphate, and ammonium chloride were injected in the caudal vein of the skate at the rate of 4 c.c. per kilo of body weight. The blood pressure and the respiratory and cardiac movements were simultaneously recorded before, during, and long after the injections.

It was found that a definite quantity of some salts stimulates, but a larger dose inhibits, or *vice versa*, one or more of the above functions. Certain salts stimulate the heart; others the respiration. The rate may be influenced separately from the force. A table was presented to show the average results of some of the experiments.

#### FURTHER RESULTS ON HETEROTRANSPLANTATION OF BLOOD VESSELS.

By C. C. GUTHRIE.

A SEGMENT of the abdominal aorta of a cat was interposed and sutured between the cut ends of a dog's carotid artery; and a similar segment from a rabbit was transplanted into another dog in the same manner.<sup>1</sup>

<sup>1</sup> GUTHRIE: This journal, 1907, xix, pp. 482-486.

After seven and one half months in the first case and eight months in the second, the circulation in each was excellent.

Upon clinical examination, marked enlargement of the segment and thickening of the wall was apparent in both.

On removal of the segment of rabbit's aorta, direct inspection confirmed the clinical findings. The lumen was greatly enlarged and free from obstruction. The total diameter of the segment was several times increased and the wall thickened. The intimal surface was smooth, glistening, and continuous with the intima of the carotid at both ends. Structural changes were marked as evidenced by a decrease in pliability, increase in transparency, and by histological examination, which, though not yet complete, showed that the transplanted segment had become more homogeneous.

Experimental studies on the nature of the physiological changes in such transplanted blood vessels are being carried on.

#### FURTHER STUDIES IN THE PHYSIOLOGY OF HEART BLOCK IN MAMMALS. CHRONIC AURICULO-VENTRICULAR HEART BLOCK IN THE DOG.

BY JOSEPH ERLANGER, JULIAN R. BLACKMAN, AND ERNEST K. CULLEN.

CHRONIC auriculo-ventricular heart block was produced in dogs either by injecting iodine into the region of the auriculo-ventricular bundle or by crushing that bundle, together with a small, variable bit of tissue in its immediate vicinity, in the clamp devised for that purpose. The operation was performed upon thirteen animals in all. Six died within twenty-four hours. Seven (Nos. 3, 4, 7, 8, 11, 12, 13) survived for from 6 to 278+ days. No. 4 had only a slight partial block which disappeared within forty-eight hours. No. 3 had at first relatively complete block from which it recovered completely in twenty-six days. Then auriculo-ventricular block was again produced by again crushing the bundle. Autopsy indicated that the first block had been caused by compression, rather than by destruction, of the auriculo-ventricular bundle and that recovery had occurred with decompression. All of the other cases had complete block, in all probability throughout life, as determined by almost daily tracings of the heart beat. Two died unexpectedly. No. 7 on the twenty-eighth, No. 9 on the ninety-second day. No. 11

died of purulent pericarditis on the sixth day. Nos. 12 and 13 are still alive and vigorous, two hundred and seventy-eight and two hundred and sixty-nine days, respectively, since the operation.

Excepting the typical features of complete auriculo-ventricular heart block, the following phenomena are of sufficient interest to warrant special mention: (1) No. 7 had at times Cheyne-Stokes respiration. With the beginning of the period of hyperpnœa there was usually marked slowing of the ventricular rate and acceleration of the auricular rate. Marked rhythmic fluctuations of the rate of the auricles alone were sometimes seen in the other cases, usually while they were resting quietly. (2) No. 11 had epileptiform seizures typical in every way of Stokes-Adams disease, and Nos. 8 and 12 had typical attacks of syncope. (3) Often a remarkable synchronism was noted between respirations and ventricular beats. (4) In cases 8 and 13 on the eightieth and seventy-fifth days, respectively, the peripheral end of the vagus was exposed and stimulated. The result was the usual inhibition of the auricles, whereas the ventricular rate was practically unaffected.

#### DISPUTED POINTS IN THE HISTOLOGY OF THE SUB-MAXILLARY GLAND AND THEIR PHYSIOLOGICAL SIGNIFICANCE.

By CHR. SIHLER (by invitation).

TEASING the gland of the kitten stained in Beale's carmine, one sees upon examining an alveolus with its adjoining capillary five kinds of nuclei: First, are darker red roundish forms, which belong to the gland cells. Second, are pale-red nuclei of oval form, which upon manipulation of the alveolus by aid of needle and coverglass can be shown to adhere to the basement membrane, and which on general principles can be looked upon as the nuclei of endothelial cells covering the membrana propria. The third form are the nuclei of the demilunes, a dark red and of demilunar form. That they are nothing else than the endothelial cells just described can be shown by flattening them out by pressing the coverglass down, sliding the needle back and forth. There is then in the gland an extensive serous cavity *within* which the capillaries are suspended. The fourth class of nuclei are those of the capillary walls, and the

fifth conspicuous nuclei on the outside of the capillaries. That these are the nuclei of the nerves supplying the gland can be shown by tracing the nerve bundles by aid of the acetic-acid-haematoxyline method, according to which small fasciculi containing one or two *nucleated* fibrils inclosed in Henle's sheath can be distinguished as the structures ready to unite with the structures they are to supply. These same *nucleated* fibrils can now be seen on the capillaries and have not been seen anywhere else. So the chorda-tympani ends at a plexus on the capillaries of the gland.

It follows that the glands are innervated by way of the capillaries. The endothelial (?) cells composing them are during nervous stimulation changed in their molecular structure or vital activity. A lymph current, different from that of the resting stage, is produced. This change in lymph supply, with modifications of pressure and chemical conditions, forms the adequate stimulus for the gland cell. This theory, however, must not be confounded with a vasomotor theory, nor does it deny any of the important functions of the gland cells.

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#### SOME OBSERVATIONS ON THE NEURO-MUSCULAR MECHANISM OF THE ALIMENTARY CANAL.

BY W. B. CANNON.

By use of a modification of the method used by Magnus the local reflex already described for the intestines was proved true of the stomach and of that part of the œsophagus having smooth muscle. This observation, together with the observation that the cardiac and pyloric sphincters obey the same reflex, indicates that the alimentary canal is a unity, made orderly by the local reflex in the myenteric plexus.

When the small intestine is stimulated simultaneously and at equal distances above and below the recording ring, by equal stimuli, the result is contraction of the recording ring — the positive effect prevails over the negative.

Evidence thus far secured points to a reversal of the local reflex in states of increasing or high tonus.

## THE PYRIMIDINE DERIVATIVES IN TRITICONUCLEIC ACID.

BY THOMAS B. OSBORNE AND F. W. HEYL.

BURIAN has raised the question whether or not the uracil and cytosine that are formed by severe hydrolysis of nucleic acid do not result from the purines, owing to the action of strong sulphuric acid in the presence of carbohydrates.

We have found that this is not the case for triticonucleic acid. The purines were separated by boiling for two hours with 2 per cent sulphuric acid and precipitating them with a hot solution of silver sulphate in 2 per cent sulphuric acid. The purine-free solution was concentrated and ammonia and total nitrogen determined in an aliquot part. It was then concentrated further until it contained 20 per cent of sulphuric acid and heated for two hours at 150°. After filtering out and washing the humus the solution contained exactly the same amount of ammonia as before, and yielded considerable quantities of cytosine and uracil, which were identified by their crystalline forms, decomposition points, and nitrogen content.

It is therefore evident that cytosine and uracil do not result from decomposition of the purines, and further that uracil was not formed from cytosine, for in either case a relatively considerable quantity of ammonia would have been found.

## OBSERVATIONS ON THE INFLUENCE OF CARBOHYDRATES ON PROTEIN METABOLISM.

BY J. R. MURLIN.

EXPERIMENTS were reported showing the effect on the protein metabolism of dogs of different amounts of cane sugar fed alone. The effect depends in large measure on the nutritive condition of the animal at the time of feeding. For example, in a dog containing a large amount of body-fat 25 per cent of the energy requirement (42 gm. of cane sugar) produced no reduction of the total nitrogen in the urine. In marked contrast with this was an emaciated dog (previously reported<sup>1</sup>), of exactly the same weight at the time of

<sup>1</sup> This journal, 1907, xx, p. 253.

feeding, in which the same amount of sugar produced a reduction of 13.3 per cent, and a pregnant dog near term, also of the same weight at the time of feeding, in which after three days of fasting the same ingestion produced a reduction of more than 30 per cent. In the fat dog the reduction with 53 per cent of the requirement (84 gm. of cane sugar) was approximately half that obtained with 110 per cent (168 gm. cane sugar).

The cane sugar produced in the fat dog a marked increase in the percentage of ammonia eliminated, the increase being greater the greater the amount ingested. There is at the same time a corresponding reduction in the percentage of urea nitrogen. In all the dogs there is an increase in the creatinin output on the sugar days. No constant change in the creatin output or in that of the undetermined nitrogen was observed.

In the pregnant dog the most noteworthy observation, aside from the great reduction in the total nitrogen produced by the sugar, was the very high excretion of preformed creatin. On the third fasting day, just previous to the sugar-feeding there were 0.264 gm. in the urine; on the two sugar days 0.441 and 0.513 gm., respectively, and on the fasting day following the sugar, 0.491 gm. The dog weighed 12 kg. This high creatin was observed also in another pregnant dog where on a third fasting day it amounted to 40 mg. per kilogram of weight.

#### ON THE LEUCOMAINS OF COD LIVER OIL.

By P. B. HAWK.

THREE samples of oil were examined, one light amber in color, another light brown, and finally a dark brown oil. The method of isolating the leucomains was essentially that suggested by Gautier and Mourgues. It is very complex and time-consuming. The leucomains were finally obtained in the form of oxalates, which were then brought into solution in water and potassium hydroxide added, whereupon there separated a rather thick oil of a brownish color. This oil was dried over potassium hydroxide and then subjected to distillation to separate the volatile from the non-volatile portion.

The weight of leucomains per kilogram of oil was as follows:

	Gm.
Light amber oil . . . . .	1.06
Light brown oil . . . . .	1.17
Dark brown oil . . . . .	1.09



The volatile portion upon being subjected to fractional distillation yielded specific volatile substances at  $87^{\circ}$ – $90^{\circ}$  C.,  $96^{\circ}$ – $98^{\circ}$  C.,  $100^{\circ}$ – $101^{\circ}$  C., and  $198^{\circ}$ – $200^{\circ}$  C. These fractions were subjected to repeated distillations, and finally the first one was found to boil at  $86^{\circ}$  C., the second at  $97^{\circ}$ – $98^{\circ}$  C., the third at  $101^{\circ}$  C., and the fourth at  $199^{\circ}$  C. Upon chemical examination these substances proved to be butylamine, amylamine, hexylamine, and dilydrolutidine.

From the non-volatile portion I succeeded in isolating and identifying the base, *morrhaine*. The other base, *aselline*, mentioned by Gautier and Mourgues, I was unable in any instance to identify.

#### A PRELIMINARY CONTRIBUTION TO THE CHEMISTRY OF THE INFUNDIBULAR PORTION OF THE PITUITARY GLAND.

By T. B. ALDRICH.

THE infundibular portion of the pituitary body (cattle) is carefully separated from the hypophysis cerebri and ground to a fine pulp. It is then heated on the steam bath with twelve times its weight of water, containing about 0.1 per cent of acetic acid, and the resulting coagulated proteid removed by filtration. The remaining proteid, together with the phosphates, is removed for the most part with uranium acetate. The filtrate is nearly colorless, usually free from proteid, and contains practically all the active blood-pressure-raising body. One cubic centimetre of such an extract, when injected intravenously, causes a rise of from 15 to 45 mm. of mercury and a pronounced slowing of the heart, depending on the sensitiveness of the dog employed; the pressure remaining above normal in some instances half an hour. One cubic centimetre of such an extract contains about 0.1 gm. of the moist infundibular portion, or 0.01 gm. ( $1/16$  grain) of the dry, and the active body must be a fraction of the latter. I think I am safe in saying that the active body causing this rise of pressure is not more than  $1/32$  gm. or less.

A crystalline picrate was obtained from the concentrated extract when aqueous or alcoholic solutions of picric acid were added to the same. Twenty-five milligrams of the dry picrate usually caused

a rise of 15 to 40 mm. of mercury. A sulphate in solution was prepared from the picrate, this being also active.

Platinic chloride also gives a double salt when added to the concentrated extract. It has not been determined whether this body is active or not.

#### A NEW CRITERION FOR THE DETERMINATION OF THE SYSTOLIC BLOOD PRESSURE WITH THE SPHYGMOMANOMETER (WITH DEMONSTRATION).

BY JOSEPH ERLANGER.

WHILE the pressure exerted on the arm by the sphygmomanometer exceeds the systolic pressure, the lever of the instrument describes upon the revolving drum waves of no inconsiderable size. When the pressure is allowed to fall slowly, this wave gradually increases in amplitude, but at the moment it falls below the systolic

pressure the wave usually suffers an abrupt increase in amplitude (method of v. Recklinghausen). At the same time the character of the motion of the lever changes. It has been found that if the drum be allowed to revolve a bit more rapidly than it is arranged to move, so that each



FIGURE 1.

wave subtends about  $1\frac{1}{2}$  to 2 mm. of smoked paper (this speed is attained merely by removing the governor), the change in form of the successive waves manifests itself usually as a more or less abrupt separation of the ascending and descending strokes of the pulse record (Fig. 1). The phenomenon may vary somewhat with the form of the pulse wave and may even be obscured by fling, but there has been no great difficulty in recognizing it in every case. It is often very clear when the tracing shows no abrupt increase in amplitude whatsoever. It is just as accurate an index to the systolic pressure as the "sensory criterion" and that of v. Recklinghausen. The change in form occurs because, at the moment the pressure on the artery falls below systolic, blood succeeds in making its way beneath the cuff. This must be squeezed

out before the lever can return to the base line, whereas at higher pressures the lever is raised only through the hydraulic ram action of the pulse wave upon the upper edge of the cuff.<sup>1</sup>

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The following communications were also presented:

FURTHER COMMUNICATION ON THE EFFECT OF VAGUS INHIBITION ON THE OUTPUT OF POTASSIUM FROM THE HEART. By W. H. HOWELL AND W. W. DUKE.

PROTEIN METABOLISM IN FASTING. By O. FOLIN.

EXPERIMENTAL GLYCOSURIA. By J. J. R. MACLEOD.

PRELIMINARY REPORT OF CERTAIN INVESTIGATIONS AS TO THE NATURE OF PEPTONES. By H. D. HASKINS.

THE SPONTANEOUS OXIDATION OF SOME CELL CONSTITUENTS. By A. P. MATHEWS.

ON THE CHEMICAL STUDY OF MENTAL DISORDERS. By WALDEMAR KOCH (read by title).

CONCERNING THE PHARMACOLOGICAL ACTION OF POTASSIUM IODIDE. By L. B. STOOKEY AND V. GARDNER.

THE CHEMISTRY OF HYPERNEPHROMAS. By H. GIDEON WELLS.

ON THE COMPOSITION OF NORMAL LYMPH FROM THE NECK LYMPHATICS OF THE HORSE. By J. R. GREER (by invitation).

THE PRESENCE OF GLUCOSE IN SALIVA. By J. G. RYAN (by invitation).

DAILY LIFE OF AMŒBA PROTEUS. By C. F. HODGE, D. GIBBS, AND O. P. DELLINGER.

THE RELATION OF PLASTICITY TO AGE IN THE DANCING MOUSE. By R. M. YERKES.

THE BACTERIO-AGGLUTINATING ACTION OF LYMPH UNDER DIFFERENT CONDITIONS OF LYMPH FORMATION. By B. BRAUDE (by invitation).

THE RELATIVE HEMOLYTIC ACTION OF SERUM AND LYMPH UNDER DIFFERENT CONDITIONS OF LYMPH FORMATION. By T. HUGHES (by invitation).

THE OSMOTIC CONCENTRATION OF THE BLOOD DURING ANÆSTHESIA. By A. B. LUCKHARDT (by invitation).

VASOMOTOR REFLEXES AFTER EXCESSIVE HEMORRHAGE. By W. T. PORTER AND H. K. MARKS.

<sup>1</sup> Compare: Johns Hopkins Hospital reports, 1904, xii, pp. 53 and 108; also This journal, 1901-1902, vi, p. xxii.

xxvi *Proceedings of the American Physiological Society.*

- ON THE MECHANISM OF THE EMBRYONIC HEART RHYTHM. By A. J. CARLSON AND W. J. MEEK.
- COMPARISON OF THE NERVOUS SYSTEM IN THE NORMAL ALBINO RAT WITH THE NERVOUS SYSTEM IN THOSE EXPERIMENTALLY STUNTED. By S. HATAI.
- OBSERVATIONS ON THE EFFECT OF EXCESSIVE TRANSFUSION OF BLOOD. By G. W. CRILE (by invitation).
- THE RELATION OF CURARA TO "NERVE ENDING" AND "RECEPTIVE SUBSTANCE." By C. W. EDMUNDS AND G. B. ROTH.
- EVAPORATION OF WATER FROM THE SKIN AND AIR PASSAGES OF MEN AT REST. By W. P. LOMBARD.
- CONCERNING THE GUAJAC REACTION. By C. L. ALSBERG.
- IONIC POTENTIAL AND TOXICITY. By A. P. MATHEWS AND R. H. NICHOLL.
- SOME POINTS IN LYMPH FORMATION. By A. J. CARLSON, J. R. GREER, AND F. C. BECHT.
- ON THE NATURE OF THE HEAT PARALYSIS IN NERVOUS TISSUES. By F. C. BECHT (by invitation).
- STUDIES IN THE RESUSCITATION OF THE CENTRAL NERVOUS SYSTEM. By F. H. PIKE (by invitation).
- FURTHER STUDIES ON THE RELATION OF THE OXYGEN SUPPLY TO THE COMPOSITION OF SALIVA. By F. C. MCLEAN (by invitation).
- THE STRUCTURE OF THE HEART MUSCLE OF LIMULUS. By W. J. MEEK (by invitation).
- OBSERVATIONS ON HUMAN CHYLE. By W. S. HALL.
- AN ATTEMPT TO DETERMINE THE MECHANISM OF PROTEIN METABOLISM IN STARVATION. By A. WOELFEL (by invitation).
- PSEUDO-FATIGUE OF THE SPINAL CORD. By F. S. LEE.
- THE TEMPERATURE COEFFICIENT OF NERVOUS CONDUCTION AS DETERMINED ON THE ISCHIADICUS OF THE FROG. By C. D. SNYDER (by invitation).
- THE RESUSCITATION OF ANIMALS KILLED BY ANÆSTHETICS (with demonstration). By G. W. CRILE (by invitation).
- THE RELATION OF ORGAN ACTIVITY TO LYMPH FORMATION IN THE SALIVARY GLANDS (with demonstration). By A. J. CARLSON, J. R. GREER, AND F. C. BECHT.
- DEMONSTRATION OF AN APPARATUS FOR THE EXTRACTION OF NERVE TISSUES. By W. KOCH.
- DEMONSTRATION OF A CO<sub>2</sub> APPARATUS. By E. P. LYON.

AN IMPROVED KYMOGRAPH. By W. T. PORTER.

DEMONSTRATION OF A MODEL SHOWING EFFECTS OF LESIONS OF HEART VALVES AND "COMPENSATION" ON THE CIRCULATION. By W. P. LOMBARD.

A NEW FORM OF ELECTRIC SIGNAL. By W. P. LOMBARD.

## RECOMMENDATIONS OF THE COMMITTEE ON PROTEIN NOMENCLATURE.

SINCE a chemical basis for the nomenclature of the proteins is at present not possible, it seemed important to recommend few changes in the names and definitions of generally accepted groups, even though in many cases these are not wholly satisfactory. The recommendations are as follows:

First: The word *proteid* should be abandoned.

Second: The word *protein* should designate that group of substances which consists, so far as at present is known, essentially of combinations of  $\alpha$ -amino-acids and their derivatives, *e. g.*,  $\alpha$ -amino-acetic acid or glycocoll,  $\alpha$ -amino-propionic acid or alanine; phenyl- $\alpha$ -amino propionic acid or phenylalanine, guanidine-amino-valerianic acid or arginine, etc., and are therefore essentially polypeptides.

Third: That the following terms be used to designate the various groups of proteins:

### I. THE SIMPLE PROTEINS.

Protein substances which yield only  $\alpha$ -amino acids or their derivatives on hydrolysis.

Although no means are at present available whereby the chemical individuality of any protein can be established, a number of simple proteins have been isolated from animal and vegetable tissues which have been so well characterized by constancy of ultimate composition and uniformity of physical properties that they may be treated as chemical individuals until further knowledge makes it possible to characterize them more definitely.

The various groups of simple proteins may be designated as follows:

— (a) *Albumins*. — Simple proteins soluble in pure water and coagulable by heat.

(b) *Globulins*. — Simple proteins insoluble in pure water but soluble in neutral solutions of salts of strong bases with strong acids.<sup>1</sup>

(c) *Glutelins*. — Simple proteins insoluble in all neutral solvents but readily soluble in very dilute acids and alkalies.<sup>2</sup>

(d) *Alcohol-soluble proteins*. — Simple proteins soluble in relatively strong alcohol (70–80 per cent), but insoluble in water, absolute alcohol, and other neutral solvents.<sup>3</sup>

(e) *Albuminoids*. — Simple proteins which possess essentially the same chemical structure as the other proteins, but are characterized by great insolubility in all neutral solvents.<sup>4</sup>

(f) *Histones*. — Soluble in water and insoluble in very dilute ammonia and, in the absence of ammonium salts, insoluble even in an excess of ammonia; yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield a large number of amino-acids among which the basic ones predominate.

(g) *Protamines*. — Simpler polypeptides than the proteins included in the preceding groups. They are soluble in water, uncoagulable by heat, have the property of precipitating aqueous solutions of other proteins, possess strong basic properties and form stable salts with strong mineral acids. They yield comparatively few amino-acids, among which the basic amino-acids greatly predominate.

## II. CONJUGATED PROTEINS.

Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(a) *Nucleoproteins*. — Compounds of one or more protein molecules with nucleic acid.

<sup>1</sup> The precipitation limits with ammonium sulphate should not be made a basis for distinguishing the albumins from the globulins.

<sup>2</sup> Such substances occur in abundance in the seeds of cereals and doubtless represent a well-defined natural group of simple proteins.

<sup>3</sup> The sub-classes defined (*a, b, c, d*) are exemplified by proteins obtained from both plants and animals. The use of appropriate prefixes will suffice to indicate the origin of the compounds, *e. g.*, ovoglobulin, myoalbumin, etc.

<sup>4</sup> These form the principal organic constituents of the skeletal structure of animals and also their external covering and its appendages. This definition does not provide for gelatin, which is, however, an artificial derivative of collagen.

(b) *Glycoproteins*. — Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid.

(c) *Phosphoproteins*. — Compounds of the protein molecule with some, as yet undefined, phosphorus containing substance other than a nucleic acid or lecithins.<sup>5</sup>

(d) *Hæmoglobins*. — Compounds of the protein molecule with hæmatin or some similar substance.

(e) *Lecithoproteins*. — Compounds of the protein molecule with lecithins (lecithans, phosphatides).

### III. DERIVED PROTEINS.

1. **Primary Protein Derivatives.** — Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations of the protein molecule.

(a) *Proteins*. — Insoluble products which apparently result from the incipient action of water, very dilute acids or enzymes.

(b) *Metaproteins*. — Products of the further action of acids and alkalies whereby the molecule is so far altered as to form products soluble in very weak acids and alkalies but insoluble in neutral fluids.

This group will thus include the familiar "acid proteins" and "alkali proteins," not the salts of proteins with acids.

(c) *Coagulated proteins*. — Insoluble products which result from (1) the action of heat on their solutions, or (2) the action of alcohols on the protein.

2. **Secondary Protein Derivatives.**<sup>6</sup> — Products of the further hydrolytic cleavage of the protein molecule.

(a) *Proteoses*. — Soluble in water, uncoagulated by heat, and precipitated by saturating their solutions with ammonium — or zinc sulphate.<sup>7</sup>

(b) *Peptoncs*. — Soluble in water, uncoagulated by heat, but not precipitated by saturating their solutions with ammonium sulphate.<sup>8</sup>

<sup>5</sup> The accumulated chemical evidence distinctly points to the propriety of classifying the phosphoproteins as conjugated compounds, *i. e.*, they are possibly esters of some phosphoric acid or acids and protein.

<sup>6</sup> The term secondary hydrolytic derivatives is used because the formation of the primary derivatives usually precedes the formation of these secondary derivatives.

<sup>7</sup> As thus defined, this term does not strictly cover all the protein derivatives commonly called proteoses, *e. g.*, heteroproteose and dysproteose.

<sup>8</sup> In this group the kyrines may be included. For the present we believe that



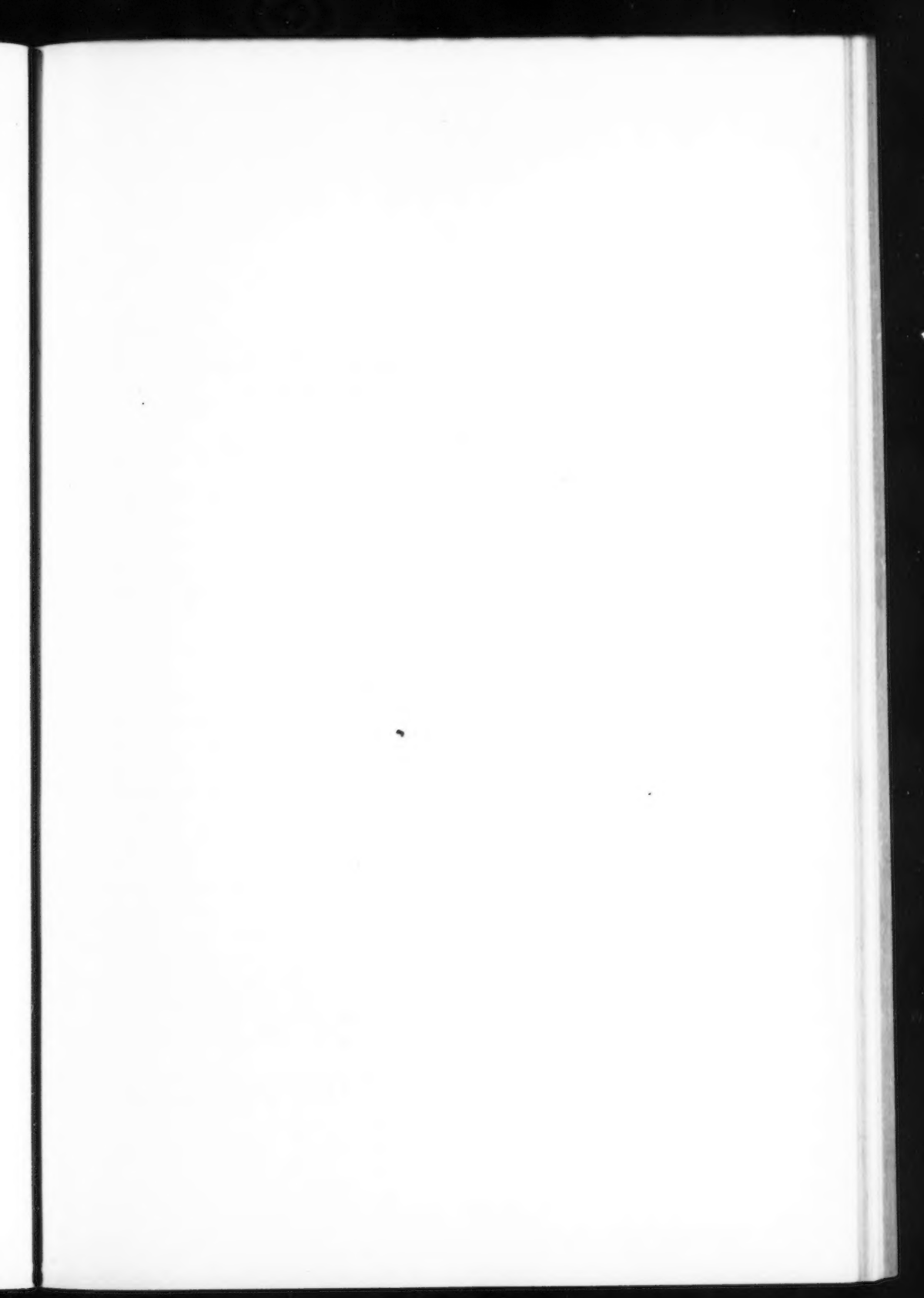
(c) *Peptides*. — Definitely characterized combinations of two or more amino-acids, the carboxyl group of one being united with the amino group of the other with the elimination of a molecule of water.<sup>9</sup>

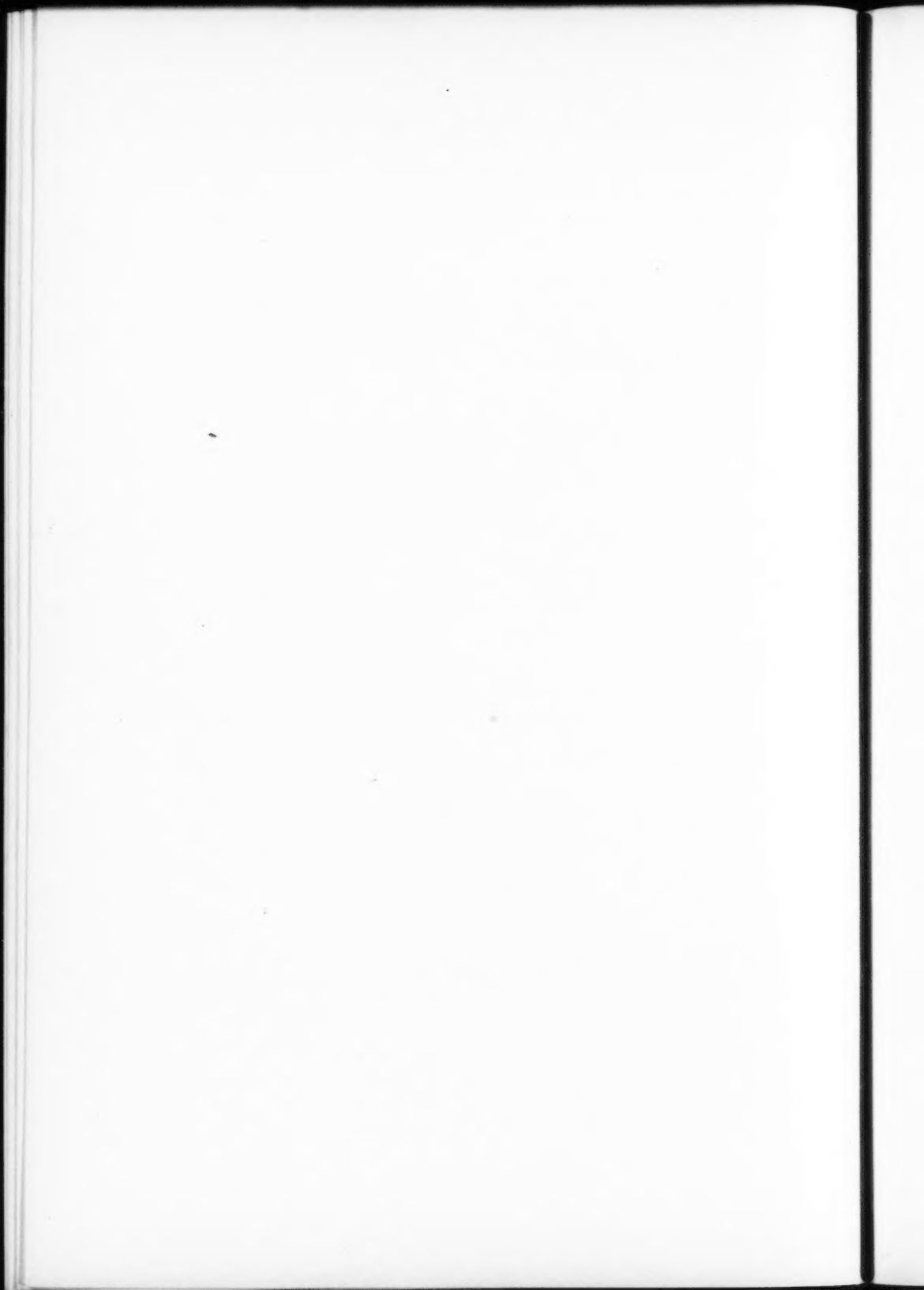
R. H. Chittenden	} <i>For the American Society of Biological Chemists.</i>	T. B. Osborne	} <i>For the American Physiological Society.</i>
Otto Folin		P. A. Levene	
W. J. Gies		J. A. Mandel	
Waldemar Koch		A. P. Matthews	
T. B. Osborne		Lafayette B. Mendel	

December, 1907.

it will be helpful to retain this term as defined, reserving the expression peptide for the simpler compounds of *definite* structure, such as dipeptides, etc.

<sup>9</sup> The peptones are undoubtedly peptides or mixtures of peptides, the latter term being at present used to designate those of definite structure.





## SOME EFFECTS OF CARDIAC NERVES UPON VENTRICULAR FIBRILLATION.

By WALTER E. GARREY.

[From the Physiological Laboratory of Cooper Medical College, San Francisco.]

THE vagus nerve of a dog with fibrillating ventricles was stimulated by faradic shocks, whereupon the normal rhythm returned promptly. The result was so striking that it seemed hardly possible that it could be merely fortuitous. The ventricles were again thrown into inco-ordinated delirium by direct faradization, and again the same result attended the stimulation of the vagus. A third attempt on the same animal was not successful. This observation convinced me that the current opinion that the vagus cannot control fibrillation is not altogether correct — that there are exceptions to the rule.<sup>1</sup> The following account deals with some experimental data which relate to these exceptions only, without affecting the validity of this view. The work which has been in progress for over two years adds to the evidence that in a certain, though small, percentage of dogs stimulation of the vagus nerve will alter the character of or even stop *ventricular fibrillation*. The *nervi accelerantes*, it was found, may upon stimulation *precipitate fibrillation*. Similar experiments with the vagus of rabbits and cats yielded results which, although significant and pointing to the same conclusions, are nevertheless far from satisfactory or positive. Even in the dog the percentage of successes is so small and the conditions so hard to obtain that one can never be certain of positive results in any given case; nevertheless, in those cases in which they were obtained I am convinced they were not mere coincidences, as has been assumed by some in criticism of the positive results of other workers in this field.

<sup>1</sup> This opinion is voiced, for example, by GASKELL in SCHAEFER's Textbook of physiology, 1898, ii, p. 192.

There is a great diversity of opinion expressed in the publications of those who have made investigations along this line. Not only is this diversity of opinion expressed by those who have worked upon ventricles, but also by those who have studied the action of nerves upon this inco-ordinated delirium of the auricles of mammals.

In the literature Knoll,<sup>2</sup> Kronecker and Spillata,<sup>3</sup> and Cushny and Edmunds<sup>4</sup> state that the vagus when stimulated has no effect upon auricular fibrillation. The last-named authors, however, make other observations, which they themselves state may have quite the opposite interpretation.

The affirmative side of this question is expressed by MacWilliam,<sup>5</sup> by Fischel,<sup>6</sup> and by Phillips.<sup>7</sup>

The observations of Cushny and Edmunds referred to above were made upon hearts which had received poisonous doses of members of the digitalis series or of barium salts. In these cases it was found that section of the vagi resulted in fibrillation of the whole heart. The effect is to be ascribed to the removal of the tonic inhibitory impulses which had been passing down the nerves holding in check any tendency to fibrillation. I have made similar observations which will be referred to later. The more recent investigations of Winterberg<sup>8</sup> also confirm the fact that fibrillation of the auricles of mammals (cats) may be "decreased or suppressed" by vagus stimulation. I have seen undoubted cases in which the auricles, which were in the wildest delirium (with marked irregularity of the ventricles such as has been described by Cushny and Edmunds [*loc. cit.*]), were stopped by vagus stimulation.

In the light of so much evidence of a positive character it must be considered as established that auricular fibrillation, at least in some of its stages, can be controlled by the vagi. I have confined my efforts more especially to the more doubtful question of ven-

<sup>2</sup> KNOLL: Archiv für die gesammte Physiologie, 1897, lxvii, pp. 587 ff.

<sup>3</sup> KRONECKER and SPILLATA: Archives internationales de physiologie, 1905, ii, p. 227.

<sup>4</sup> CUSHNY and EDMUNDS: American journal of the medical sciences, 1907, cxxxiii, p. 66.

<sup>5</sup> MACWILLIAM: Journal of physiology, 1887, viii, p. 296.

<sup>6</sup> FISCHEL: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xxxviii, pp. 228 ff.

<sup>7</sup> PHILLIPS: Archives internationales de physiologie, 1905, ii, p. 271.

<sup>8</sup> WINTERBERG: Archiv für die gesammte Physiologie, 1907, cxvii, pp. 223 ff.

tricular fibrillation. Concerning this question of the control by the vagi of ventricular delirium, it is not surprising that we should find a much greater uncertainty pervading the literature of the subject when we consider the fact that the vagus normally has much less influence upon the ventricles than upon the auricles; in fact it has even been questioned whether the vagus does really innervate this part of the heart at all.<sup>9</sup>

This doubt cannot be entertained, however, in the light of the work of many other investigators,<sup>10</sup> who have shown an unquestionable innervation of the ventricles by the vagus nerves in certain cases, although it is undoubtedly of less physiologic efficiency than that of the auricles. This view finds corroboration in the experiments which I shall present in this paper. That such a question should be raised may be more readily accounted for when we consider that various individuals of a given species, dogs in particular, show marked variations in this regard. In considering the action of the vagus it is hardly necessary to refer to the differences which exist among different species of mammals, — the slight tonic inhibition in the rabbit, for instance, as compared with the dog, or the relatively slight action of the cat's vagus when stimulated in comparison with that exhibited in either of the other two animals.

The following investigators failed to note effects of the vagus upon fibrillating ventricles: Hoffa and Ludwig;<sup>11</sup> Vulpian;<sup>12</sup> S. Mayer.<sup>13</sup> See Bochefontaine and Roussy,<sup>14</sup> MacWilliam,<sup>15</sup> and Winterberg.<sup>16</sup>

The following authors, on the contrary, record positive results:

<sup>9</sup> ERLANGER and HIRSCHFELDER: *Zentralblatt für Physiologie*, 1905, xix, p. 270, and *This journal*, 1906, xiv, p. 153.

<sup>10</sup> MACWILLIAM: *Journal of physiology*, 1888, ix, p. 167, and *Ibid.*, p. 357; HERING: *Archiv für die gesammte Physiologie*, 1905, cviii, p. 281; RIHL: *Ibid.*, 1906, cxiv, p. 545; HOWELL and DUKE: *Journal of physiology*, 1906, xxxv, p. 145.

<sup>11</sup> HOFFA and LUDWIG: *Zeitschrift für rationelle Medizin*, 1849, ix, p. 107. Quoted by WINTERBERG.

<sup>12</sup> VULPIAN: *Archives de physiologie*, 1874, p. 975.

<sup>13</sup> MAYER: *Sitzungsberichte der Akademie der Wissenschaften zu Wien*, 1873, lxviii, p. 74. Quoted by WINTERBERG.

<sup>14</sup> See BOCHEFONTAINE and ROUSSY: *Comptes rendus*, 1881, xcii, p. 87.

<sup>15</sup> MACWILLIAM: *Journal of physiology*, 1887, viii, p. 296.

<sup>16</sup> WINTERBERG: *Archiv für die gesammte Physiologie*, 1907, cxvii, p. 223.

Einbrodt,<sup>17</sup> Bezold,<sup>18</sup> and Laffont.<sup>19</sup> Bayliss and Starling<sup>20</sup> also record the fact that ventricular fibrillation is less easily induced while the vagi are being stimulated, and Cushny and Edmunds (*loc. cit.*) have found that by vagus stimulation "the ventricle is rendered slower and more regular or may be arrested completely," an effect which they ascribe simply to the production of a blocked heart. The fact, however, that the ventricles came to a *complete standstill* while the auricles in their cases continued fibrillating shows not only a blocked heart, but also a possible innervation of the ventricles by the vagi.

Another observation which has, I believe, a direct bearing upon the affirmative side of this question has been made by MacWilliam (*loc. cit.*); namely, that a heart may be thrown into fibrillar movement by the injection of a solution of atropin and subsequently arrested by pilocarpin, complete recovery of the ventricle taking place. The relation of these drugs to the vagus innervation is too well known to call for further comment.

The diversity in the results recorded by these two groups of investigators is not surprising to one who has worked in this field and knows its difficulties and uncertainties. Negative results may easily be accounted for by the normally slight action of the vagus upon the ventricles. That more positive results are not recorded I believe is due simply to the fact that few have systematically investigated this field, our literature being made up largely of purely incidental observations. Furthermore, when the conditions necessary to the eliciting of a positive action of the vagus upon cardiac fibrillation are more fully understood, I am convinced we shall have more records of a positive nature.

In the first experiments of my series rabbits, anæsthetized either with chloral or urethane, and some ether when necessary, were used, but it was soon learned that the results were untrustworthy, principally owing to the great difficulty of inducing a lasting fibrillation. This condition may, to be sure, follow as a result of direct faradization of the ventricles, but far more frequently the delirium ceases spontaneously, and not infrequently there was an abrupt

<sup>17</sup> EINBRODT: Sitzungsberichte der Akademie der Wissenschaften zu Wien, 1859, xxxviii, p. 345. Quoted by WINTERBERG.

<sup>18</sup> BEZOLD: Untersuchungen aus dem Laboratoriums zu Würzburg, 1867, i, p. 256.

<sup>19</sup> LAFFONT: Comptes rendus, 1887, cv, p. 1092.

<sup>20</sup> BAYLISS and STARLING: Journal of physiology, 1892, xiii, p. 407.



cessation of all inco-ordination as soon as the electrodes were removed from the ventricle. One cannot therefore be certain beyond any reasonable doubt that the cessations are due to nerve stimulation, and not to the inherent peculiarities of the heart in its recovery from this condition.

In spite of this uncertainty my experiments made upon me a strong impression that in this animal ventricular fibrillation induced by direct stimulation is more difficult to produce if the vagus nerves are simultaneously stimulated. It is further certain that of those instances in which there was an abrupt stop of delirium when the stimulating shocks were stopped, the larger proportion were cases which had been subjected simultaneously to vagus stimulation which was continued after that of the ventricles was stopped, with the result that fibrillation ceased abruptly, the whole heart being thrown into diastolic rest, after which a normal although accelerated rhythm was resumed when the vagus stimulation was discontinued. Another impression which was strong, but which the nature of the cardiac action in this animal did not permit of reducing to an absolute or satisfactory demonstration, was that the fibrillation of the rabbit's ventricle may be induced in a shorter time with induction shocks of a given strength, and with weaker shocks if the vagus has previously been cut. If this view could be substantiated, it would controvert the current idea that the vagi possesses no tonic inhibitory action upon the heart of the rabbit. It was the impression gained from the more or less positive character of these results which induced me to continue the investigation upon other animals.

Before proceeding with the consideration of further experiments I wish to record the results upon the cardiac rhythm when the rabbit's ventricle is directly faradized. Immediately surrounding the tips of the stimulating electrodes there is a local area of inco-ordination. If the stimulating shocks are weak, this area is very circumscribed, and remains so, the rest of the heart beating in a perfectly co-ordinated way; but there is a most striking acceleration of rate, the auricle as well as the ventricle being involved in this change. The rhythm in these cases is a reversed one, that is, of the ventriculo-auricular type described by MacWilliam. This is readily demonstrated when the heart is first brought to a vagus standstill and the ventricle then stimulated. When the strength of the stimulating faradic shocks is gradually increased, we note a larger area of inco-ordination surrounding the electrodes, and an

accelerated rate of co-ordinated rhythm of both ventricle and auricle. Further increase in the strength of the faradic shocks, or even moderately strong but long-continued stimulation, results in wild delirium of the ventricles, and occasionally of the auricles also. So long as there is any semblance of co-ordination in the ventricular contractions, the auricles seem able to respond to every contraction, but promptly, on onset of ventricular fibrillation, we find that in addition to auricular acceleration there is an absolute irregularity of the auricle, indicating, again, that the beat is a reversed one, and that the stimuli arising from the irregularly acting ventricles are being transmitted across the auriculo-ventricular bridge irregularly. A condition which stimulates this closely may be induced in the turtle's heart, while the ventricles are being faradized, the auricles showing a marked acceleration and great irregularity, both in strength and rate of contractions. If during this condition a clamp be screwed tightly down upon the auriculo-ventricular ring, or the Stannius' ligature be applied, the auricular irregularity disappears, and the rate changes abruptly to normal, although the ventricles are kept in continued fibrillation by induction shocks. This experiment demonstrates further that the auricles were not being affected by any escape of the stimulating current.

Another fact of significance, and pointing to the same conclusion, is the following: If, while the electrodes are producing the fibrillary condition of the ventricles, the vagus be also stimulated, the auricles frequently come to rest in diastole, while the ventricles continue fibrillating so long as strong faradic shocks are being applied to them. This is true not only for rabbits, but is also true for dogs, even in those cases of persistent ventricular fibrillation which cannot be controlled by the vagus. It has been mentioned that sometimes the auricle as well as the ventricle fibrillates when the latter has been stimulated, and I have also noted the reverse when the auricle has been stimulated, in both classes of animals just referred to. I have never seen this take place, however, if the vagus nerve was being stimulated; and so far as negative experiments are of any value, they certainly point to an inhibiting action upon both auricles and ventricles, or else to so marked a decrease in the conductivity of the auriculo-ventricular bridge as to prevent the spread of the fibrillary condition. The importance of these experiments upon the reversal of the rhythm is at once seen when we note that the results coincide exactly with

those of Cushny and Edmunds (*loc. cit.*), except that they are ventriculo-auricular in type instead of auriculo-ventricular, — results which have been applied by these authors in a most significant way to the explanation of certain cases of paroxysmal tachycardia in man.

When cats were used as experimental material, the results were very similar to those obtained with rabbits, with the exception that it was found to be easier to produce a lasting ventricular fibrillation in this animal than in the rabbit, although less so than in the dog. The results of vagus stimulation were, on the whole, not more certain than in the rabbit, although in three out of eleven animals fibrillation, apparently firmly established, gradually changed its character and then ceased abruptly while the vagus was being stimulated. One should hardly expect a very striking result from the stimulation of this nerve in cats, for even the normal beat is only slowed by this means, and only occasionally is it possible to bring the heart to a complete standstill.

When dogs are used as experimental material, it is found that the ventricles are easily thrown into fibrillation, a condition which is very persistent, rarely disappearing spontaneously, — a fact which is important for the interpretation of my experiments, inasmuch as it minimizes the possibility of mere chance as a factor in the phenomena observed.<sup>21</sup>

In my investigations upon this animal it soon developed that the results depended in large measure upon its physical condition. When anæsthetized with ether and worked upon immediately, no satisfactory results could be obtained, and in those cases in which success was obtained the animals were in a decidedly abnormal condition, — a condition which served the purposes of this investigation most admirably, however. The first success, which has already been referred to, was with a dog which had served for other physiological experimentation under inefficient artificial respiration for about two hours. The dog had previously been anæsthetized with a large dose of morphine. At the time of the fibrillation experiment the condition of the animal was as follows: Rectal temperature, 34.2° C.; mean blood pressure, about 50 mm. of mercury. The heart had been exposed to the air for a long

<sup>21</sup> Fibrillation of the auricles is much more difficult to obtain than that of the ventricles, and moreover much more frequently disappears spontaneously. Can this be due to the more pronounced influence of the vagus upon the auricles?

time, and was beating at a rate of only sixty per minute, in spite of the fact that the vagus nerves had been cut, so that the slow rate was not due to the asphyxiated condition of the central nervous system. The abnormal condition of the heart was further indicated by the length of the auriculo-ventricular pause, which was even more pronounced than in cold-blooded animals, and by the fact that not infrequently the ventricles failed to respond to auricular contractions, indicating a partially blocked heart, — a condition which had not existed at the time the chest was opened. This condition was approximated in all of the experiments which were attended with any marked degree of success; many normal animals were worked with before it was appreciated that this abnormal condition might be an important factor in the results, no success ever having been noted in animals which were anaesthetized and worked with immediately. The successful experiments were furthermore obtained only with young animals. The actual determining factors in these conditions have not been ascertained, although the following facts should be considered, for they all have some place in the general condition which we have outlined. Hough<sup>22</sup> states that the vagus has a greater effect than normal upon a weakened heart. Suschtsinsky<sup>23</sup> has shown that a low blood pressure increases the action of the vagus, and Danielewski has found that asphyxia has a similar effect. Carbon-dioxide and cold are among the factors used by Porter<sup>24</sup> to decrease the tendency to fibrillation. All of these factors must be taken into consideration in any statement of the causes underlying the successful attempts to control fibrillation by stimulation of the vagus nerve. No matter what conditions may be present in an experiment, it is certain that only a small percentage of animals will show this positive control of fibrillation of the ventricles by the vagus nerve.

I shall now proceed to an analysis of the successful experiments, grouping together changes of a similar character, or describing single experiments, as seems best to convey an adequate idea of the different effects which the vagus may have upon fibrillation of the ventricles.

First. I wish to record three cases in which, *after* opening the

<sup>22</sup> HOUGH: *Journal of physiology*, 1895, xviii, p. 161.

<sup>23</sup> SUSCHTINSKY: *Zeitschrift für die medicinischen Wissenschaften*, 1868, No. 3.

<sup>24</sup> PORTER: *This journal*, 1898, i, p. 71, and *Journal of experimental medicine*, 1896, i, p. 46.

chest and pericardium, *section of the vagi high up in the neck induced fibrillation* of the whole heart. This observation is the same as that made by Cushny and Edmunds (*loc. cit.*) after poisoning with digitalis, and indicates that the abnormal condition which prevailed in my animals, and which has just been considered, was probably not unlike that induced by the members of this series of drugs. In all three of these cases the section of one vagus alone failed of this result, which, however, followed promptly when the other vagus was sectioned. I concur in the view of the above authors that in these cases the removal of vagus inhibition is the factor responsible for the onset of fibrillation. The fact that the whole heart goes into delirium, and not simply the auricle, also points strongly to a vagus control of the ventricles, as well as of auricles, although it does not exclude the possibility of the effect being a sequence of the auricular fibrillation. In one of these cases subsequent stimulation of the left vagus had no effect on the cardiac delirium, although the right vagus when stimulated stopped the fibrillating heart in diastole,<sup>25</sup> there being no visible fibrillation, although the previous inco-ordination returned after the stimulation had stopped. The vagus stimulation was repeated a second time upon the same heart with the same result. In the other two cases stimulation of the vagi separately or together had no effect upon the course of the delirium cordis.

Second. It is more difficult to induce *ventricular* fibrillation by faradic shocks if the vagus nerve is simultaneously stimulated. Bayliss and Starling found this to be true; Winterberg, however, denies it and, in fact, records just the opposite results. My results coincide with those of the former investigators; for instance, it was found, in Experiment 32 of my series, that when the two stimulations, of the musculature and of the nerve respectively, were simultaneously applied, the only effect upon the ventricle, provided the shocks were not too strong, was that of local inco-ordination immediately under and around the electrodes, with a marked acceleration of the rate of contraction of the whole heart. When the ventricular stimulation was removed, the heart came to a diastolic standstill, owing to the inhibitory effect of the vagus stimulation; it began to beat, but not to fibrillate, when the electrodes were again applied to the ventricle. Subsequent removal of the

<sup>25</sup> This difference between the two vagi I have repeatedly observed upon the normal dog, although it is not a constant physiologic feature in this animal.

electrodes from the vagus, while those on the ventricle remained, resulted in fibrillation of the ventricle. This is a conclusive demonstration that in these cases the vagus had inhibited the onset of the abnormal contractions.

In conducting these experiments care was taken to start the coils of the inductorium, which is used for stimulating the ventricle, widely separated, and gradually to approximate them to the point at which the above result was obtained; for if too strong a stimulation of the ventricles be resorted to at the outset, they will fibrillate in spite of the vagus influence. This fact, however, cannot be construed as signifying that the vagus does not exert an inhibitory influence upon fibrillation, any more than the fact that in spite of vagus stimulation a stimulus directly applied to the cardiac musculature calls forth a contraction can be construed as indicating that the vagus normally can exert no inhibitory action whatever.

One point was developed in this investigation which might lead to an erroneous conclusion and to the assumption that the vagus stimulation actually increased the liability to fibrillation; namely, it was found that it was necessary to remove the electrodes which were being directly applied to the ventricle before stopping the vagus stimulation, otherwise fibrillation might ensue, and this is true even with faradic shocks of a strength which fail to induce fibrillation of the ventricles when the heart is beating co-ordinately; in other words, in these cases the actual vagus influence had been removed before fibrillation began. It may be conceived, in explanation of this phenomenon, that the vagus action has left the ventricular musculature in a more excitable condition or with a greater tendency to fibrillation owing to decreased conductivity or increased excitability resulting possibly from the lowered blood pressure and lack of coronary circulation; however that may be, it seems more probable that it is a manifestation of the fact that the quiescent heart is more readily thrown into delirium than one which is beating regularly,—a fact which I have repeatedly observed. The fact of itself in no way invalidates our conclusion, for *while* the vagus was exerting its inhibitory action upon the heart it required a stronger stimulus (induction shocks) applied directly to the ventricle to call out the fibrillation. Other authors (Cushny and Edmunds) have seen a heart go into fibrillation immediately after stimulation of the vagus was stopped. This observation falls, I am convinced, into the same category with the facts we have just



been discussing. The fact that in their cases fibrillation did not take place while the vagus was being stimulated shows that this procedure did not in and of itself precipitate the fibrillary state, but in the light of my experiments rather proves the opposite; namely, that a heart with a naturally strong tendency to fibrillate was prevented from doing so by the tonic inhibitory action of the vagus nerves and by their artificial stimulation, but that in the interval of rest following the stimulation the inco-ordinated movements were easily instituted spontaneously, possibly because the ventricles were at rest and because there had been no coronary circulation for a considerable interval.

In spite of these findings relative to the action of the vagus upon fibrillation of the ventricles, it is not inconceivable that under other conditions vagus stimulation may have other effects upon the inco-ordination, even to the extent of actually precipitating it. Such variability in results would not in the least disagree with our knowledge of the ways in which the vagus affects the cardiac musculature, especially that of the auricle. A suppression of conductivity alone while the auricles are being directly stimulated, for example, might conceivably result in a condition in which the different parts of the heart are in different phases of rapid contraction simultaneously, that is, are in fibrillation which, without vagus stimulation, would be only a marked irregularity. On the other hand, with a fibrillating musculature a decrease in excitability and rhythmicity such as the vagus might induce would result in a cessation of fibrillation. Winterberg's observations (*loc. cit.*) offer some support to this view of a twofold action of the vagi, for, as noted above, he found that auricular fibrillation could be suppressed by vagus influence; his experiments, however, also show other conditions in which fibrillation seemed to be a result of vagus stimulation.

In our own laboratories Dr. A. W. Hewlett has made some interesting observations upon auricular fibrillation (not yet published) which, coupled with my own experiments, lend support to this view. He has found that an auricle which is being thrown into marked irregularity by direct faradization sometimes passes into the fibrillary state when faradic shocks are applied to the vagus nerves. In all of my experience I have never seen a case in which stimulation of the vagus *alone* precipitated cardiac fibrillation, and it seems certain that it is only when coupled with some other factor, such



as a heightened excitability of the musculature or its simultaneous stimulation, that this vagus effect can be elicited; even in this latter case the opposite result may ensue, as outlined above.

The action of the accelerator nerves is a factor which must be taken into consideration, as will be pointed out below.

Third. *The vagi may simply alter the character of the fibrillation of the ventricles without abolishing it.* In this group of cases fibrillation was first started by direct faradization of the ventricles, and the stimulating electrodes at once removed (the auricles, as usual, beat rapidly and irregularly, as has been described for the rabbit's heart); next a cut vagus was stimulated, whereupon the auricles came promptly to a standstill.<sup>26</sup> And the ventricular fibrillation was seen to change gradually from a fluttering delirium to a series of undulating sluggish movements, the progress of the waves over the surface being easily followed with the eye. Only direct observation can give any adequate idea of these changes, for the registering apparatus fails to make any record of their character; the height of the tracing may even be increased, giving to the uninitiated the idea that the movements are actually increased, whereas the opposite is the true status, the contractions in reality approximating a co-ordinated condition and giving small carotid pulse waves. This condition of undulation may be made to persist for some time if the stimulation of the vagus is continued. When the vagus stimulation is stopped in these cases, the ventricles return to the original condition of violent delirium; the auricle too again becomes very irregular. This result of vagus stimulation has been repeated two, three, or even four times in the same experiment, but ultimately the action of the vagus on the fibrillating ventricles fails to appear, although the auricles are still inhibited in each instance. This is but an expression of the fact that a heart which has fibrillated for a long time (or repeatedly) is less amenable to vagus control and more easily thrown into fibrillation a second or third time, — facts which I have noted repeatedly.

The changes which we have just discussed are to a certain extent a function of the intensity of stimulus applied to the vagus. Thus it was seen that a stimulus of an intensity just sufficient to inhibit

<sup>26</sup> This indicates either a marked decrease in the excitability of the auricles resulting from vagus stimulation, or else it indicates the production of a ventriculo-auricular blocked heart, for the latter factor may have no inconsiderable share in this result. A similar result obtained by CUSHNY and EDMUNDS during auricular fibrillation has already been referred to.

the auricles in no case had any perceptible effect upon the process of ventricular fibrillation; those changes which we have just described were induced only by completely approximating the coils of the inductorium. It happened frequently, however, as I have noted, that even the strongest faradic shocks had no effect whatever upon the course of the ventricular fibrillation.

Fourth. It is the nearest step from this group to that in which the control of ventricular fibrillation by the vagus is absolute. The method is that just described, and we find that with the strongest vagus stimulation the inhibitory changes of fibrillation are the same as those just recorded, and in fact may go no farther if the excitation be of short duration; but with continuation of vagus stimulation these changes may be carried a step farther, the ventricles as well as the auricle, although considerably later, being reduced to a state of diastolic quiescence. Upon removal of the inhibitory vagal influence the whole heart took up a normal co-ordinated rhythm and did not return to the condition of delirium. This result was obtained with six animals. In three of these cases I succeeded in repeating the experiment a second time on each animal, but a third attempt was successful in only one case. It thus develops that repeated or long-continued fibrillation of the ventricle renders it less amenable to vagus influence, although the auricles could still be inhibited. It is therefore necessary to make the vagal stimulation in these cases as soon after inception of fibrillation as possible if one would have any assurance of success. I have been fortunate in making this a class demonstration. One case has previously been referred to (p. 291) in which only the right vagus affected the fibrillation; in two of the three cases just considered, however, that is, in which it was possible to repeat the stimulation of the vagus and show an inhibition of the ventricular inco-ordination, these nerves were both tested and both found equally effective. Furthermore it was found that the later stages of fibrillation could not be controlled by either of the vagi or when both were simultaneously stimulated. Inasmuch as both ventricles were in a fibrillary state in these experiments, it would appear that each of the nerves has a distribution affecting the whole heart.

Fifth. The suggestion has been made that the control of the ventricles of the dog by the vagi is only apparent, and that in reality the cessation of beats in a normal animal is due to the fall of intraventricular pressure consequent on the cessation of auricular

beats. My experiments do not substantiate this view, for I find that both exsanguinated and excised hearts will respond to the inhibitory influences of the vagi and come to rest in diastole. In harmony with this fact it should not be overlooked that when the *whole heart* is in fibrillation the blood-pressure changes which result from the delirium are of such a nature that the inhibition of auricles can have little effect upon intraventricular or coronary pressures, and we must conclude that where ventricular fibrillation is affected by the vagi, the results are due to the fact that the ventricles themselves are innervated. In order to be more certain of this conclusion I repeated my experiments in such a manner that the effects of vagus stimulation when blood-pressure changes were excluded could be determined. To this end ligatures were passed about both venæ cavæ of a large dog, with a consequent drop in intracardiac and arterial pressures. Fibrillation of the ventricles alone was then induced by strong faradic shocks, the vagi were cut, and the left vagus immediately stimulated; the auricles at once came to a standstill, but the ventricular inco-ordination seemed unaffected. I then stimulated the left vagus, which stopped the whole heart in diastole with no return of the fibrillation. The vena cava was next incised, the incision extending into the auricle, and the aorta was likewise cut, with complete exsanguination of the heart. The ventricles were again thrown into fibrillation by direct faradization; stimulation of the right vagus for several seconds again stopped the whole heart, which beat co-ordinately although very feebly when released from the inhibitory influence. An attempt to repeat the experiment upon this heart resulted in failure, and fibrillary death of the heart ensued. In this experiment the changes cannot be ascribed to changes of blood pressure, but point to the direct innervation of the ventricle as well as to the control of fibrillation by the vagus nerves. In this connection the following experiment was performed upon the excised heart. The dog had been morphinized on two successive days, and etherized to complete anæsthesia. The vagus nerves were cut and dissected the whole length of the neck. After cutting the abdominal aorta and vena cava to get rid of the future bleeding, the thoracic viscera along with the cardiac nerves were rapidly removed; the heart was exsanguinated and washed off externally with alkaline Ringer's solution, and then suspended from a hook attached to the aortic wall. The beats had come to a standstill

by the time the necessary manipulation was completed. The right vagus nerve was then stimulated continuously by faradic shocks, and electrodes from a second inductorium were directed against the ventricles. A few isolated beats resulted, but no generalized fibrillation until the electrodes were removed from the vagus, when the ventricles fibrillated and induced irregular beats of the auricle. This state continued although the electrodes were also removed from the ventricle. Now stimulation of the right vagus brought the whole heart to a standstill, and the resting condition persisted when the vagus stimulation was stopped. Mechanical or faradic stimuli applied to the auricles resulted in co-ordinated beats or groups of beats involving the whole heart. Repetition on the same heart was unsuccessful, and four other attempts with excised hearts of dogs were also unsuccessful. No question of blood-pressure changes can possibly enter as a factor in this result. The relation of the vagus to the fibrillation of the ventricles is especially striking in these cases, owing to the rarity of spontaneous recovery under such conditions.

If we turn now to a consideration of the effects of the accelerans upon fibrillation, we find a very meagre literature indeed. Reid Hunt<sup>27</sup> records that he frequently obtained fibrillation of the ventricle, but not of the auricle, when he stimulated the dog's accelerans, and H. E. Hering<sup>28</sup> noted an occasional increase in ventricular fibrillation upon stimulation of this nerve. Winterberg,<sup>29</sup> on the other hand, concludes that these nerves have no effect either upon the onset or the course of ventricular fibrillation. He comes to this conclusion in the face of the fact that his experiments in reality gave the opposite result. This conclusion will be considered later. The accelerans had been repeatedly stimulated in the course of my general experiments, and I have at present a record of four instances in which ventricular fibrillation was undoubtedly induced by this means. In none of these cases did the auricles also fibrillate, although they beat very irregularly (not regularly, as Reid Hunt described). One of these cases was carefully tested to determine whether, in regard to fibrillation, antagonism between the vagus and accelerans exists. Complete success attended this effort. The heart was exsanguinated by dividing

<sup>27</sup> HUNT: This journal, 1899, ii, p. 421.

<sup>28</sup> HERING: Zentralblatt für Physiologie, 1905, xix, p. 132.

<sup>29</sup> WINTERBERG: *Loc. cit.*

the abdominal aorta and vena cava, to remove all possibility of blood-pressure changes and still to leave a clean thorax with nerves intact. (a) The vagi were cut, and the right vagus and left accelerans (prepared in Reid Hunt's method, by resection of the first two ribs) were then simultaneously stimulated. The heart continued to beat, but unfortunately no record was taken of the type or rate of the contractions. Both stimuli were then removed from the nerves, the heart continuing to contract normally. This experiment, in conjunction with what follows, corroborates Hunt's finding, that the vagus and accelerans are antagonists, and that upon simultaneous stimulation the result will be the arithmetical mean of the individual effects. (b) Strong stimulation of the accelerans alone now induced fibrillation. The stimulation was continued, and the right vagus also stimulated. This, however, had no perceptible effect, as both nerves were being stimulated, the fibrillation of the ventricles continuing violently. (c) The next stage of the experiment consisted in a removal of the accelerans stimulation, while that of the vagus was continued. The result was a gradual change in type, followed by the sudden cessation of fibrillation and a diastolic standstill of the heart, which afterward took up a normal rhythm when the inhibitory action of the vagus ceased. There were only a few co-ordinated beats, however, in this case, the heart coming to rest almost at once. Gentle mechanical stimulation, however, always resulted in a co-ordinated contraction or group of contractions, showing that the heart was really in a normal resting condition and not in one of reduced or masked fibrillation. The experiment could not be repeated upon this heart, except in its first stage; namely, that the ventricles did not go into fibrillation when the two nerves were simultaneously stimulated, but did when the vagus stimulation was subsequently stopped, that of the accelerans being continued. It would be impossible for one to picture mentally a more perfect example of the mutual antagonism of these two nerves, or of the fact that both nerves may act upon the ventricles, or, further, of the fact that *fibrillation may be subject to the same nervous influences as the normal modes of cardiac contraction*. This experiment, further, furnishes the strongest kind of evidence that the accelerans must be looked upon as a potential motor cardiac nerve. This view has also been advanced upon other evidences by Hering<sup>30</sup>

<sup>30</sup> HERING: Zentralblatt für Physiologie, 1905, xix, p. 129, and Archiv für die gesammte Physiologie, 1906, cxv, p. 354.

and by Howell and Duke.<sup>31</sup> Guthrie and Pike<sup>32</sup> also state that they started quiescent hearts by stimulating the accelerans.

Winterberg, I have stated, found an action of the accelerator nerve similar to that outlined above, fibrillation being the result of its stimulation, but he believed the effect was an indirect one upon the coronary circulation, — an assumption which is entirely gratuitous. In my experiments the section of the abdominal blood vessels eliminated the coronary circulation from consideration, for there was none. An indirect effect through the vagus is also precluded by the previous section of those nerves.

I believe it is hardly possible, in the face of the experiments which have been presented in the foregoing sketch, to assume that the positive results which have been recorded in the literature by different competent observers were mere coincidences. In the cases which I have presented there have always been some factors which apparently rule out any such chance results. The fact, however, cannot be overlooked that *these nervous influences upon fibrillation are not by any means general*, in fact could never be obtained upon a normal animal, and in my series could be satisfactorily demonstrated in only about 16 per cent of those animals in which I had duplicated conditions which seemed most favorable for the demonstration of the phenomena. The further fact develops that there are varying degrees of fibrillation, some of which may be partially or completely controlled by the vagus nerve, while others are wholly beyond any nervous influence. This is evidenced by the fact that in all my experiments hearts previously affected by the vagus, if they had fibrillated long or repeatedly, finally entered this uncontrollable condition. This condition, however, was amenable to Hering's potassium chloride treatment.

#### SUMMARY.

1. Proof of vagus control of ventricular fibrillation could only be found in dogs, although rabbits and cats showed slight indications of some influence of the vagus upon this condition.
2. Those dogs which gave positive results were all in a very abnormal state, and only in small percentages of these animals could the positive result be obtained.

<sup>31</sup> HOWELL and DUKE: *Journal of physiology*, 1906, xxxv, p. 144.

<sup>32</sup> GUTHRIE and PIKE: *This journal*, 1907, xviii, p. 27.



3. Cutting of both vagi may sometimes precipitate cardiac fibrillation, affecting both auricles and ventricles.

4. Stimulation of the vagi may make the induction of ventricular fibrillation more difficult.

5. Fibrillation of the ventricles once started may (although infrequently) be changed in character or entirely stopped by vagus stimulation; sometimes only one vagus, sometimes both nerves, showing this control.

6. This control of ventricular fibrillation may be obtained independently of the blood-pressure changes due to stopping of the auricles, *e. g.*, in exsanguinated and excised hearts, indicating a direct vagal innervation of the ventricles in these cases.

7. The ventricles, but not the auricles, in this series of experiments are sometimes thrown into fibrillation by stimulation of the accelerans.

8. The vagus and accelerans are direct antagonists in their effects upon fibrillation.

9. In every case in which the vagus showed any control of fibrillation the ventricle could ultimately be thrown into a violent state of inco-ordination which was entirely beyond the influence of any nerve stimulation.



## GLUCOSE IN SALIVA.

BY A. J. CARLSON AND J. G. RYAN.

[From the Hull Physiological Laboratory of the University of Chicago.]

SALIVA is commonly held to be free from sugar. So far as we have been able to learn, the literature contains no records of the presence of sugar in the saliva of any mammal in normal condition. Normal human saliva gives none of the tests for sugar, but there are records of the presence of sugar in the saliva of diabetic persons. Fleckseder<sup>1</sup> reports sugar in two cases of diabetes mellitus, the sugar being constantly present in mixed saliva in amounts readily detected by the Fehling's test. Mosler,<sup>2</sup> on the other hand, reports a number of cases of diabetes mellitus in which the parotid saliva failed to give positive reactions for sugar with any test. We have had the opportunity of testing the mixed saliva of a case of diabetes in man, whose urine sugar usually ran above  $3\frac{1}{2}$  per cent. The phenylhydrazine test and the Fehling's reduction tests were negative, the safranin test positive. It would therefore seem that even in the case of pronounced hyperglycemia in man, sugar may or may not appear in the saliva, and when the sugar does appear in the saliva it is only in slight traces.

In the course of experiments on some points in the secretion of ptyalin our attention was called to a reducing substance present in considerable quantities in the submaxillary and parotid saliva of the cat collected during ether anæsthesia. At first we were led to believe that the cat's submaxillary and parotid saliva contains a considerable quantity of ptyalin, but it was soon discovered that the greater part of the reduction of the Fehling's solution in the starch-saliva mixtures was due to a reducing substance in the saliva itself. This substance turned out to be glucose. It is present in considerable quantity in saliva collected during anæsthesia and is

<sup>1</sup> FLECKSEDER: *Centralblatt für innere Medicin*, 1905, xxvi, p. 41.

<sup>2</sup> MOSLER: *Archiv für Heilkunde*, 1864, v, p. 228.

found in traces in normal reflex saliva. The greater concentration of the sugar in the saliva secreted during anæsthesia appears to be due to hyperglycemia invariably induced by the anæsthetic.

## I. METHODS.

The mixed reflex saliva was obtained by stimulation of the mucous membrane of the mouth with ether vapor or weak acetic acid, the mouth being first thoroughly washed with water so as to remove possible traces of sugar or reducing substances. The cats have to be held down more or less forcibly in this process, and most of them struggle considerably when the ether vapor touches the mouth. This may tend to bring on hyperglycemia, and thus may account for the sugar appearing in the saliva. However, at least two of our cats were docile and struggled very little during the process of securing the saliva; nevertheless the saliva of those cats gave positive tests of sugar.

In a number of experiments we secured mixed saliva from the mouth after hypodermic injections of pilocarpin. By this method the saliva was obtained practically without any struggle on the part of the animals. This saliva also gave positive tests for sugar.

In order to remove all possibility of the reducing substance in the saliva coming from the mouth cavity itself, cannulas were inserted in the ducts of Stenson under local anæsthesia. Pure parotid saliva was thus secured by the reflex stimulation methods mentioned above or by hypodermic injections of pilocarpin. This pure parotid saliva gave positive tests for sugar.

In order to determine the relation of the concentration of the sugar in the blood to the concentration of the sugar in the reflex saliva, hyperglycemia was induced by injection of glucose. These injections were made hypodermically into a muscle, or intravenously, the femoral vein being isolated under local anæsthesia.

The submaxillary chorda and sympathetic salivas were always collected under light ether anæsthesia.

## II. RESULTS.

1. *The cat's submaxillary and parotid salivas contain a reducing substance or substances.*—The normal or reflex saliva was tested

in seven cats. In each case the safranin test was positive. The fermentation test was positive in the four cases in which it was used. Five cases gave slight reduction with Fehling's solution, while osazone crystals were obtained in only one case. These experiments are summarized in Table I.

TABLE I.

TESTS SHOWING THE PRESENCE OF SUGAR IN THE NORMAL SALIVA OF THE CAT.

No. of exp.	Saliva.	Safranin.	Fermentation.	Reduction with Fehling's solution.	Phenylhydrazine.
I.	Reflex mixed saliva . . . .	+	+	+	—
II.	Reflex mixed saliva . . . .	+	+	+	+
III.	Reflex mixed saliva . . . .	+	+	+	—
IV.	Reflex mixed saliva . . . .	+	+	+	—
V.	Reflex mixed saliva . . . .	+	no test	—	—
VI.	(a) Reflex mixed saliva . . .	+	no test	+	—
	(b) Clear parotid saliva (temporary fistula) . . . .	+	no test	—	—
VII.	(a) Reflex mixed saliva . . .	+	no test	+	—
	(b) Clear parotid saliva (temporary fistula) . . . .	+	no test	+	—

TABLE II.

SHOWING THE INCREASE IN THE SUGAR CONTENT OF NORMAL SALIVA AND PILOCARPIN SALIVA AFTER INTRAVENOUS AND HYPODERMIC INJECTION OF GLUCOSE. CAT. NO ANÆSTHESIA.

No. of exp.	Saliva, mixed.	Safranin.	Fermentation.	Reduction with Fehling's solution.	Phenylhydrazine.
I	1. Normal reflex saliva . . .	+	no test	—	—
	2. Reflex saliva after intravenous injection of glucose.	+	+	+	+
	3. Pilocarpin saliva following 2	+	+	+	+
II	1. Normal reflex saliva . . .	+	no test	—	—
	2. Reflex saliva, after hypodermic injection of glucose. .	+	+	+	+
	3. Pilocarpin saliva following 2	+	+	+	+

The submaxillary saliva secured by direct stimulation of the secretory nerves and the submaxillary and parotid saliva secured by pilocarpin under general ether anaesthesia contains this reducing substance in greater amounts than the normal or reflex saliva. Tests were made on salivas from six cats under ether anaesthesia, all of them yielding positive reaction with phenylhydrazine, safranin, Fehling's solution and fermentation. One typical experiment of this series is summarized in tabular form in Table III.

2. *The reducing substance in the cat's saliva is glucose.* — Our first thought was that the reduction was due to the mucin in the saliva, as it is well known that, on splitting, mucin yields a carbohydrate body that reduces Fehling's solution. We removed the mucin from the saliva by precipitation with acetic acid. The saliva free from mucin still gave positive tests for sugar. Safranin, moreover, is not discolored by mucin, and in our hands this test was always positive. Although this possibility seems to be strengthened by the fact that the submaxillary saliva always contains more of this reducing substance than the parotid saliva, just as the former contains more mucin than the latter, the above facts excluded mucin as a factor in the reduction. Moreover the concentration of the reducing substance does not run parallel with that of the mucin in the saliva.

The sugar is fermentable. The osazone crystals obtained were those typical for glucose. We made melting-point determinations of these crystals from salivas of three cats. In one case the crystals melted at  $198^{\circ}\text{C}.$ ; in the other two cases the liquefaction occurred between  $200^{\circ}\text{C}.$  and  $204^{\circ}\text{C}.$  The melting-point of pure glucosazone crystal is  $204^{\circ}\text{--}205^{\circ}$ .

The slightly lower figures in our experiments are in all probability due to impurities adhering to the crystals. The figures are nevertheless too high for pentosazones. The sugar must be a hexose, in all probability glucose.

3. *The glucose in cats' saliva is not a special product of the salivary glands, but is simply the glucose of the blood eliminated by the glands,* in much the same manner as the kidneys eliminate sugar in hyperglycemia. This is shown by the following facts: (1) Hyperglycemia induced by injection of glucose solution hypodermically or intravenously is invariably followed by a great increase in the reducing substance in the saliva. This is true both for normal or reflex saliva as well as for the saliva secured under anaesthesia

TABLE III.

DETAIL OF ONE EXPERIMENT ON THE CONCENTRATION OF GLUCOSE IN THE CAT'S SALIVA COLLECTED DURING ETHER ANÆSTHESIA.

Gland.	Saliva.	Safranin test.	Fermentation test.	Reduction with Fehling's solution.	Phenylhydrazine test.
Submaxillary, right.	1. Chorda . . . . .	+	+	+	+
	2. Chorda . . . . .	+	+	Λ	Λ
	3. Chorda . . . . .	+	+	Λ	Λ
	4. Chorda . . . . .	+	+	Λ	Λ
	5. Chorda after 30 min. rest	+	+	Λ	Λ
	6. Pilocarpin . . . . .	+	+	Λ	Λ
	7. Pilocarpin . . . . .	+	+	Λ	Λ
Parotid, right.	1. Pilocarpin . . . . .	+	—	+	+
	2. Pilocarpin . . . . .	+	+	Λ	Λ
Submaxillary, left.	1. Chorda . . . . .	+	+	+	+
	2. Chorda . . . . .	+	+	Λ	Λ
	3. Chorda . . . . .	+	+	Λ	Λ
	4. Pilocarpin . . . . .	+	+	Λ	Λ
	5. Pilocarpin . . . . .	+	+	Λ	Λ
	6. Pilocarpin . . . . .	+	+	Λ	Λ
Parotid, left.	1. Pilocarpin . . . . .	+	+	+	+
	2. Pilocarpin . . . . .	+	+	Λ	Λ
	3. Pilocarpin . . . . .	+	+	Λ	Λ
Sublingual, right.	1. Chorda . . . . .	+	+	+	+
	2. Chorda and pilocarpin .	+	+	Λ	no test.
Sublingual, left.	1. Corda and pilocarpin .	+	—	no test.	+
Urine, at end of experiment . . .		+	no test.	+	+

by stimulation of the secretory nerves or by pilocarpin. The concentration of the reducing substance in the saliva varies directly with the degree of hyperglycemia produced.

(2) It has already been stated that there is a greater concentration of the sugar in the saliva collected during anæsthesia than in normal reflex saliva. The cause of this difference is probably the following. Ether anæsthesia in the cat invariably induces hyperglycemia and diabetes during the course of the anæsthesia. This is shown by the appearance of sugar in the urine and by an increase in the percentage of sugar in the blood. The urine collected from five cats killed by decapitation gave no reduction test for sugar. Normal cat's urine is therefore practically free from sugar. The urine drawn from the bladder of cats after one or two hours of ether anæsthesia invariably yields great reduction and masses of osazone crystals. And this diabetes is probably caused by hyperglycemia, because after thirty to sixty minutes of ether anæsthesia the blood contains a much greater percentage of sugar than at the beginning of the anæsthetic. The case of the increased sugar content of saliva during anæsthesia is therefore not to be sought primarily in a change in the permeability or secretory activity of the gland, but in the increased concentration of the sugar in the blood.

4. *There is a gradual increase of glucose in the successive samples of saliva secreted during anæsthesia.*—This is true both for the parotid and the submaxillary salivas. This point was discovered in trying to determine whether the saliva sugar is produced in the glands themselves. If quantities of 1 c.c. of chorda saliva be collected during one to two hours of anæsthesia, the last sample contains much more sugar than the first; in fact each succeeding sample contains more sugar than the preceding one. In some of our experiments as many as seven to ten samples were secured from each gland.

This gradual increase in sugar content appears also in the saliva produced by the injection of pilocarpin and is equally marked in the submaxillary and the parotid glands.

The cause of this progressive increase in sugar content of the saliva may be (1) a gradual increase in the sugar content of the blood, or (2) a gradually increasing permeability or alteration in the secretory activity of the gland. The first alternative is probably the true explanation. We have seen that the percentage of sugar in the saliva bears a direct relation to the percentage of sugar in

the blood. Ether produces hyperglycemia. This is in all probability due to an increased elimination of sugar by the liver. This conversion of liver glycogen into sugar is a progressive one; hence we have a gradual increase in the sugar content of the blood during the anæsthesia.

That it is not a question of a gradual increase in the permeability of the glands seems to be shown by the fact that the gradual increase in the sugar content is not obtained when the hyperglycemia is produced suddenly by direct injection of glucose into the veins. In that case there is, of course, no *gradual* increase in the sugar of the blood.

5. *The parotid saliva contains a smaller percentage of glucose than does the submaxillary saliva.*—In none of our experiments did we observe an exception to this rule. Only a few tests were made on the saliva from the sublingual gland, but these indicated that the sugar content of the sublingual and the submaxillary salivas is about the same.

This difference between the submaxillary and parotid saliva indicates that the sugar does not pass into the saliva as a result of filtration or being carried along passively in the water stream. If such was the case, we would expect more sugar in the serous saliva (parotid) than in the mucous saliva (submaxillary). However, the cats' submaxillary saliva does not contain a great deal of mucin.

6. *The submaxillary saliva produced by stimulation of the cervical sympathetic contains more sugar than that secured by stimulation of the chorda tympani.*—This is best brought out by alternately stimulating the chorda and the sympathetic on the same side. Three or four sympathetic samples of 1 c.c. each and as many chorda samples may thus be obtained for comparison from each side. It will not serve to compare the sympathetic saliva from one gland with the chorda saliva from the gland of the opposite side, because the corresponding glands in the same animal may not yield the same quantity of sugar in the saliva. When the comparisons are made between alternate samples of chorda and sympathetic saliva from the same gland, the sympathetic saliva is always found to contain the greater amount of sugar. In some cases the chorda saliva collected after a period of sympathetic stimulation contained more sugar than the sample collected prior to the stimulation.



We have at present no adequate explanation to offer for this phenomenon. Langley discovered long ago that the sympathetic submaxillary saliva of the cat is more dilute than the chorda saliva, and this has been confirmed by Carlson, Greer, and Becht. The greater dilution seems to be due to the smaller quantity of organic constituent which is present in greater amount than in chorda saliva. It is therefore probable that the difference between the organic contents of the sympathetic and the chorda saliva noted by Langley is mainly due to the mucin.

Carlson<sup>3</sup> has shown that the cervical sympathetic in the cat contains both vaso-dilator and vaso-contractor fibres to the submaxillary gland, and that the usual effect of stimulation of the sympathetic with the weak interrupted current is vaso-dilation in the gland. The increased percentage of sugar in the sympathetic saliva cannot therefore be related to partial anæmia of the gland from vaso-constriction. It was pointed out by Carlson, Greer, and Becht<sup>4</sup> that the difference in composition of cats' sympathetic and chorda submaxillary saliva cannot be due to differences in vascular conditions of the gland, but must be due to a difference either in the nature of the action or in the regional distribution of the two nerves in the gland. These two alternatives cover the present case also, but we have no evidence that will enable us to decide in favor of the one or the other.

### III. SUMMARY.

1. The normal saliva (submaxillary, sublingual, parotid) of the cat contains a trace of glucose. This glucose is probably not a specific product of the salivary glands, but simply the glucose of the blood transferred into the saliva by the gland cells.

2. The percentage of glucose in the saliva increases with an increase in the percentage of glucose in the blood. The glucose is present in greater quantity in the saliva collected during the anæsthesia; this is probably due to the hyperglycemia that is invariably produced by the anæsthetic. But the condition of the gland is also a factor in determining the percentage of the glucose, as the chorda saliva collected simultaneously from the left and the

<sup>3</sup> CARLSON: This journal, 1907, xix, p. 408.

<sup>4</sup> CARLSON, GREER, and BECHT: This journal, 1907, xx, p. 180.

right submaxillary glands may not contain the same percentage of the sugar.

3. The saliva from the submaxillary gland always contains more sugar than the parotid saliva. And in the case of the submaxillary saliva itself that secured on stimulation of the chorda tympani contains less sugar than the sympathetic submaxillary saliva. We have no adequate explanation for these variations.

## FURTHER STUDIES ON RESISTANCE TO LACK OF OXYGEN.

By WALES H. PACKARD.

[From the Marine Biological Laboratory at Woods Hole, and the Biological Department of the Bradley Polytechnic Institute, Peoria, Ill.]

### I. INTRODUCTION.

IN a previous paper<sup>1</sup> it has been shown that the effect of those carbohydrates which can be absorbed when injected into the body cavity of the common minnow, *Fundulus heteroclitis* (*i. e.*, maltose, glucose, and lævulose), is to increase the resistance of these animals to lack of oxygen. This effect of these sugars was explained on the assumption that they act as depolarizers in the processes of protoplasmic respiration, uniting with the nascent hydrogen formed in these processes and thus permitting oxidation to go on in the absence of air.

The observations described in the following paper are an extension of the experiments already published. They were undertaken to test the effect of the other food substances and some drugs, when injected intraperitoneally, on the resistance to lack of oxygen.

The same animals (*Fundulus heteroclitis*) were used, and the experiments were performed in a similar manner to that described in the previous paper,<sup>2</sup> which may be briefly outlined as follows. The fish were first assorted according to size and sex and were then injected into the body cavity with three to eight drops, according to the size of the animal, of the solution whose effect was to be tested. After injection they were replaced in running water to allow time for absorption. The definite lengths of time in each experiment are stated in the tables. For an experiment, ten of the injected animals and ten others of the same size properly marked

<sup>1</sup> PACKARD: This journal, 1907, xviii, p. 164.

<sup>2</sup> PACKARD: *Loc. cit.*

to serve as controls were placed in a litre flask which was then completely filled with sea water and tightly stoppered. Under these conditions the available supply of oxygen was quickly exhausted and the animals were under conditions of lack of oxygen. The length of time the animals were left in the flask in each experiment is given in the tables. It varies somewhat with the temperature. If the animals were not left in the flask long enough, they would all revive, and if left too long, they would all be dead. The animals were then removed from the flask and placed in running water for several hours, until all those not actually killed in the experiment had revived.

In all experiments of this character the importance of large numbers of individuals is to be strongly emphasized. The individual variation in resistance to lack of oxygen is so great that one experiment or a few experiments may show nothing. It is only when large numbers are used that the effect sought for can be demonstrated. In the following tables all the experiments which were performed are given.

## II. THE EFFECT OF MANNOSE AND GALACTOSE ON RESISTANCE TO LACK OF OXYGEN.

The earlier observations on the effect of carbohydrates on resistance to lack of oxygen were extended to include two other monosaccharides, mannose and galactose. The results of the experiments on injection with mannose are shown in Table I.

From the summary it will be seen that out of 126 alive individuals 88, or 70 per cent, were those which had been injected, while only 38, or 30 per cent, were the controls. Of the 134 dead individuals only 42, or 31 per cent, were injected, while 92, or 69 per cent, were controls.

If the mannose had no effect on the resistance to lack of oxygen, an approximately equal number, or 50 per cent each, of injected and controls would have been left alive and an equal number would have been dead.

The results show that a much greater percentage of injected individuals than of the controls were left alive and a correspondingly smaller percentage of the injected than of the controls were dead. The effect of the injection of mannose is therefore to in-

crease the resistance to lack of oxygen and is similar to the effect of the glucose and lævulose.

Table II shows the results of injection with galactose. It will be seen from the summary that an approximately equal number of

TABLE I.  
FUNDULUS INJECTED WITH 0.5 MOL. MANNOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
28 00	2 45	7	3	3	7
27 45	2 45	6	4	3	7
27 30	2 45	7	3	6	4
27 20	2 45	6	4	4	6
18 25	2 45	8	2	2	8
18 20	2 45	5	5	2	8
18 10	2 45	9	1	3	7
23 30	2 45	6	4	2	8
23 15	2 45	9	1	3	7
22 25	2 30	6	4	2	8
22 10	2 30	5	5	3	7
22 05	2 30	8	2	3	7
22 00	2 30	6	4	2	8
Total . . . . .		88	42	38	92

SUMMARY.

Alive, 126 . . . . . Injected, 88 (70 per cent). Controls, 38 (30 per cent).  
Dead, 134 . . . . . Injected, 42 (31 per cent). Controls, 92 (69 per cent).

injected animals and controls were left alive and a correspondingly equal number were dead. The difference in percentage between the injected individuals and controls both alive and dead is well within the limits of individual variation and would probably disappear if larger numbers were used. The injection of galactose therefore has no effect on the resistance to lack of oxygen and is

similar to that found in previous experiments for cane sugar and lactose.

TABLE II.  
FUNDULUS INJECTED WITH 0.5 MOL. GALACTOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
26 50	2 45	6	4	4	6
26 35	2 45	3	7	3	7
26 25	2 25	10	0	5	5
26 10	2 45	6	4	8	2
17 25	2 45	9	1	4	6
22 45	2 45	4	6	6	4
16 20	2 45	3	7	3	7
15 35	2 45	5	5	3	7
20 55	2 30	5	5	8	2
20 50	2 30	7	3	4	6
23 15	2 30	2	8	1	9
22 15	2 30	2	8	3	7
16 5	2 45	1	9	3	7
22 50	2 30	0	10	2	8
15 50	2 45	1	9	1	9
23 00	2 30	0	10	0	10
22 30	2 30	1	9	1	9
Total . . . . .		65	105	59	111

SUMMARY.

Alive, 124 . . . Injected, 65 (52 per cent). Controls, 59 (48 per cent).

Dead, 216 . . . Injected, 105 (48 per cent). Controls, 111 (52 per cent).

The fact that the disaccharides, cane sugar and lactose, did not increase the resistance to lack of oxygen, as did maltose, was explained<sup>3</sup> as probably due to the lack of the necessary enzymes for

<sup>3</sup> PACKARD: *Loc. cit.*

converting them into the simple sugars, as it is generally recognized that the disaccharides must be converted into monosaccharides before they can be absorbed and assimilated. These disaccharides would be found rarely if ever in the food of fishes, and the absence of the inverting enzymes would receive a teleological explanation in the statement that the specific enzymes are developed only in response to the requirements of the individual animal. Thus lactase has been found very generally wanting in animals lower than the mammals and also in the adults of many of the mammals.<sup>4</sup> The absence of a lactase would not, however, explain the fact that the injection of galactose does not produce any increase in resistance to lack of oxygen, for it is already a monosaccharide and does not require inversion before it is supposedly capable of absorption. There is, however, some evidence that galactose is not assimilated to any great extent. Thus Blumenthal<sup>5</sup> found that the limit of assimilation of galactose after intravenous injection in rabbits was very low, being but slightly above that of cane sugar or lactose and far below that of dextrose or *lævulose*. Pavy<sup>6</sup> also, in his studies on the intravenous injection of carbohydrates in rabbits, reports that while all the monosaccharides were utilized to a great extent (*i. e.*, not eliminated in the urine), yet galactose was utilized to a less extent than dextrose and *lævulose*.

On the other hand, Dastre<sup>7</sup> found that, after subcutaneous and intravenous injection, galactose appeared only as traces in the urine and was therefore utilized to almost as great an extent as was either dextrose or *lævulose*.

Fritz Voit<sup>8</sup> found that, after subcutaneous injection in the human subject, galactose was not recovered from the urine, but was absorbed similarly to dextrose, *lævulose*, and maltose. McGuigan,<sup>9</sup> who with Dr. S. A. Matthews determined the amount of various sugars it is necessary to inject intravenously in rabbits before the

<sup>4</sup> See MENDEL and MITCHELL: This journal, 1907, xx, p. 80, for a short summary of the literature on the presence and distribution of the inverting enzymes and a bibliography.

<sup>5</sup> BLUMENTHAL: Beiträge zur chemischen Physiologie, 1905, vi, p. 829. The original paper was not available. Cited from MENDEL and MITCHELL: This journal, 1905, xiv, p. 239.

<sup>6</sup> PAVY: Journal of physiology, 1899, xxiv, p. 479.

<sup>7</sup> DASTRE: Centralblatt für Physiologie, 1889, p. 133.

<sup>8</sup> VOIT, F.: Deutsches Archiv für klinische Medicin, 1897, lviii, p. 523.

<sup>9</sup> MCGUIGAN: This journal, 1907, xix, p. 175.



appearance of sugar in the urine, reports that galactose was more readily oxidized than either l  vulose or dextrose.

While the evidence in mammals is thus conflicting, there is no doubt, from the experiments given in this paper, that in fishes at least galactose is not absorbed to any great extent when injected intraperitoneally, for if the galactose had been absorbed it would probably have increased the resistance to lack of oxygen, since McGuigan<sup>10</sup> has found that, outside the body, galactose is oxidized by cupric acetate more easily than dextrose and only slightly less easily than l  vulose.

This failure to absorb galactose in fishes might receive the same teleological explanation given above in the fact that lactose or its splitting products would ordinarily never occur in their food.

### III. THE EFFECT OF FATS ON RESISTANCE TO LACK OF OXYGEN.

Since it has been shown that carbohydrates when they can be absorbed increase the resistance of fishes to lack of oxygen, and also that proteids<sup>11</sup> when fed to the fish have no such effect, there is left yet to be considered the third group of food substances, the fats, which were next tested as to their effect on resistance to lack of oxygen. With this end in view the *Fundulus* were fed into the stomach by means of a pipette with 2-3 c.c. of oil and then placed in running water until the next day, or even longer (the definite lengths of time are shown in the tables), to allow time for the digestion and absorption of the oil, which would probably be rather slow. Both olive oil and linseed oil were used, and the results are given in Tables III and IV.

It will be seen from the summaries that in each case an approximately equal number of controls and fed individuals were left alive and an equal number were dead, thus showing that under the conditions of the experiment no effect on resistance to lack of oxygen was obtained. It was seen, on dissecting some of the fed individuals, that the oil had disappeared from the stomach and intestines, but whether it had been digested and absorbed or whether it had been passed out as excreta could not be determined, as the animals were necessarily left in running water and any oil excreted would have been immediately washed away.

<sup>10</sup> MCGUIGAN: *Loc. cit.*

<sup>11</sup> PACKARD: *Loc. cit.*

The experiment was then tried of injecting linseed oil into the body cavity of the fish. The animals were left as before until the next day to allow time for absorption. The results are shown in Table V.

TABLE III.  
FUNDULUS FED WITH OLIVE OIL.

Time between feeding and placing in flask.	Time in flask.	Fed.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
20 10	2 30	3	7	3	7
24 00	2 30	2	8	2	8
22 45	2 30	5	5	4	6
39 20	2 40	4	6	5	5
39 00	2 40	5	5	2	8
24 00	2 30	3	7	6	4
23 50	2 30	4	6	4	6
23 35	2 30	8	2	6	4
23 20	2 30	3	7	3	7
38 20	2 40	1	9	4	6
Total . . . . .		38	62	39	61

SUMMARY.

Alive, 77 . . . Fed, 38 (50 per cent). Controls, 39 (50 per cent).  
Dead, 123 . . . Fed, 62 (50 per cent). Controls, 61 (50 per cent).

It will be seen from the summary that under the conditions of the experiment the resistance to lack of oxygen was greatly decreased. A much larger percentage of the injected animals than of the controls were dead, while a smaller percentage of the injected than of the controls were left alive.

An explanation of this result has not yet been arrived at. A series of fish were injected with linseed oil in a manner similar to that in the experiment and were then left in an aquarium for more than two weeks. During that time none of the fish showed any deleterious effect of the injected oil. At the end of that time all

the fish were alive and active and apparently normal. On opening the body cavity of the fish at the end of the two weeks it was found that it was still filled with the injected oil, and it could not be easily determined by comparing the amount with that previously injected whether any oil had been absorbed during all that time or not. It

TABLE IV.  
FUNDULUS FED WITH LINSEED OIL.

Time between feeding and placing in flask.	Time in flask.	Fed.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
8 15	2 45	4	6	3	7
8 00	2 25	5	5	5	5
21 15	2 45	5	5	3	7
20 45	2 45	5	5	3	7
22 30	2 30	4	6	5	5
22 15	2 30	5	5	4	6
18 35	2 30	5	5	6	4
18 20	2 30	6	4	9	1
18 00	2 30	4	6	6	4
Total . . . . .		43	47	45	45

SUMMARY.

Alive, 88 . . . Fed, 43 (49 per cent). Controls, 45 (51 per cent).  
Dead, 92 . . . Fed, 47 (51 per cent). Controls, 45 (49 per cent).

was judged that it had not. Since, then, the oil is probably not absorbed from the body cavity, no importance with reference to its effect on respiration can be attached to this experiment.

#### IV. THE EFFECT OF ALCOHOL ON RESISTANCE TO LACK OF OXYGEN.

The extensive literature on the physiological effects of alcohol both as a drug and as a food was thoroughly reviewed a few

years ago.<sup>12</sup> The earlier experiments on the influence of alcohol, considered as a drug, on respiration are concerned principally with the effect on the frequency and depth of the respiratory movements and with the influence on the oxygen intake and carbon-dioxide output. Although the earlier data are somewhat conflict-

TABLE V.  
FUNDULUS INJECTED WITH LINSEED OIL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
24 30	3 00	4	6	3	7
24 00	3 00	1	9	5	5
23 45	3 00	1	9	7	3
23 30	3 00	5	5	5	5
22 30	3 00	3	7	5	5
22 00	3 00	4	6	7	3
24 10	2 15	3	7	8	2
24 40	2 30	4	6	6	4
24 25	2 30	0	10	3	7
Total . . . . .		25	65	49	41

SUMMARY.

Alive, 74 . . . . . Injected, 25 (33 per cent). Controls, 49 (67 per cent).  
Dead, 106 . . . . . Injected, 65 (62 per cent). Controls, 41 (38 per cent).

ing, yet the later results, as summarized by Abel,<sup>13</sup> seem to indicate that in the higher animals, including man, alcohol in moderate doses acts as a respiratory stimulant for an hour or more after administration. This stimulating effect is seen in a slight increase in the volume of air passing through the lungs and in an increase in the absorption of oxygen (3.5 per cent). The stimulating effect,

<sup>12</sup> J. S. BILLINGS, Ed.: *Physiological aspects of the liquor problem*, 1903.

<sup>13</sup> ABEL: *Pharmacological action of ethyl alcohol; Physiological aspects of the liquor problem*, ii, p. 116.

however, is very transitory and is soon followed by a depressant action on both the rate and depth of the respiratory movements, an effect which is always obtained with the use of larger doses of alcohol. The explanation given by Singer<sup>14</sup> is adopted as the most probable one: "It is well known that alcohol dilates the superficial blood vessels and thus leads to an increase in heat dissipation. The animal organism counteracts this loss by an increase in heat production. In other words, alcohol induces loss of heat from the body and at the same time causes a compensatory increase in the oxygen intake in order that this loss may be made good by an increased combustion." Alcohol is thus not a direct respiratory stimulant, but acts only indirectly through an increased demand for oxygen required by an increase in the oxidative processes in the tissues which in turn was brought about by an increase in heat dissipation.

The question whether or not alcohol had a direct effect on protoplasmic respiration was not considered.

In summing up the literature on alcohol considered as a food in its relation to respiration, Atwood<sup>15</sup> gives the following:

1. "There is no evidence that alcohol, in moderate quantities, has any effect upon oxygen absorption different from that of ordinary nutrients.

2. "There is no evidence that alcohol in moderate quantities has any effect upon the oxidation of carbon or upon the elimination of carbon-dioxide, different from that of ordinary nutrients."

Atwater also calls attention to the fact that a clear distinction must be drawn between alcohol as a food and alcohol as a drug.

In his own experiments on the nutritive value of alcohol in which the "respiration calorimeter" was used, and which were far more accurate in plan and execution than any others which had preceded, Atwater<sup>16</sup> reaches the same conclusion, namely, that alcohol in moderate quantities (72 gm. of absolute alcohol per day) is oxidized in the body like sugar, starch, and fat and thus serves as a fuel, and that the body burns the alcohol at its disposal in the same way as any other food in sufficient quantity to supply the energy it needs for warmth and work. In other words,

<sup>14</sup> SINGER: Physiological aspects of the liquor problem, ii, pp. 112 *et seq.*

<sup>15</sup> ATWOOD: The nutritive value of alcohol; Physiological aspects of the liquor problem, ii, p. 190.

<sup>16</sup> ATWATER: Physiological aspects of the liquor problem, ii, p. 292.

alcohol in this quantity acts as a food and interferes in no way with the consumption of oxygen and the production of carbon-dioxide.

Since in general the experiments already mentioned do not touch directly the question as to the effect of alcohol on protoplasmic respiration, it was determined to test the effect of alcohol on resistance to lack of oxygen as bearing more directly on this point.

In the first few preliminary experiments the results obtained were very irregular, a fact which has been noted by all investigators on the effects of alcohol. There is evidently a great individual variation in the sensitiveness of different individuals to the effects of alcohol, and consistent results can be obtained only by the use of many experiments and large numbers of individuals. It was determined, therefore, to make a series of experiments with gradually increasing strengths of alcohol; commencing with 1 per cent and increasing until a strength was reached which was as much as the animals would stand. With each per cent of alcohol at least eight experiments were made involving the use of two hundred animals. In all about one hundred experiments were performed with more than two thousand fish, and the results showed a gratifying consistency. The alcohol used was the ordinary commercial alcohol (96 per cent), and was diluted with distilled water to make the proper per cent. The animals were injected with from three to eight drops of the alcohol according to the size of the fish. In each experiment only animals of approximately the same size were used, and an effort was made in the different experiments to inject an amount proportional to the size of the animals used, so that in all the experiments on each strength of alcohol, as near as possible, approximately the same ratio of alcohol to body weight was used. From ten to fifteen minutes were allowed after the injection of the alcohol for absorption before the animals were placed in the flask.

The results of all the experiments with the injection of alcohol are summarized in Table VI. In this table are collected together the summaries of individual tables similar in form to Tables VII-IX, but which are not given here.

The distilled water used in the dilution of the alcohol was first injected in a series of experiments as a control, and a summary of the results is included in the table with the results of the alcohol. The distilled water is seen to be without any effect on resistance to lack of oxygen, as an equal number (50 per cent)

of both controls and injected were left alive and an equal number (50 per cent) were dead. Hence, whatever effect on resistance to lack of oxygen was obtained with the diluted alcohol may be attributed to the alcohol itself, and not to the water used in dilution. From the remainder of the table it will be seen at a glance

TABLE VI.  
FUNDULUS INJECTED WITH ALCOHOL.<sup>1</sup>

Strength of alcohol injected.	Alive.			Dead.		
	Total.	Injected.	Controls.	Total.	Injected.	Controls.
Dist. H <sub>2</sub> O	69	34 (50%)	35 (50%)	71	36 (50%)	35 (50%)
1 per cent.	95	44 (47%)	51 (53%)	85	46 (54%)	39 (46%)
2 " "	85	40 (47%)	45 (53%)	55	30 (55%)	25 (45%)
3 " "	74	31 (42%)	43 (58%)	86	49 (57%)	37 (43%)
4 " "	71	28 (40%)	43 (60%)	89	52 (57%)	37 (43%)
5 " "	103	43 (42%)	60 (58%)	97	57 (58%)	40 (42%)
10 " "	99	37 (37%)	62 (63%)	141	83 (59%)	58 (41%)
15 " "	58	18 (31%)	40 (69%)	102	62 (60%)	40 (40%)
20 " "	96	28 (29%)	68 (71%)	64	52 (81%)	12 (19%)
25 " "	90	25 (27%)	65 (73%)	70	55 (79%)	15 (21%)
30 " "	93	25 (26%)	68 (74%)	67	55 (82%)	12 (18%)

<sup>1</sup> In these experiments with alcohol the summaries only of the tables, and not the individual tables, are given. With each per cent of alcohol at least eight experiments were made.

that with every strength of alcohol used there is a decrease in resistance to lack of oxygen, and that in general the resistance to lack of oxygen decreases in proportion as the strength of the alcohol used increases. The percentage of injected individuals left alive gradually and steadily decreases from 50 per cent when distilled water was used to 26 per cent when 30 per cent alcohol was used, and the percentage of injected individuals which were dead steadily increases from 50 per cent with distilled water to 82 per cent with 30 per cent alcohol, while there is a correspond-



ing increase and decrease in the percentage of controls left alive and dead. This decrease in resistance is very slight with 1 and 2 per cent alcohol, where it is almost within the limits of experimental error, but still it is a decrease and not an increase. With 3 per cent and higher strengths the resistance rapidly decreases.

There are certain small irregularities which a careful examina-

TABLE VII.  
FUNDULUS INJECTED WITH 10 PER CENT ALCOHOL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
2 30	2 05	5	5	7	3
2 30	2 05	2	8	3	7
1 40	2 00	7	3	8	2
2 30	2 00	6	4	5	5
2 45	1 30	5	5	7	3
2 40	1 30	8	2	10	0
2 15	1 45	6	4	7	3
2 10	1 45	8	2	8	2
Total . . . . .		47	33	55	25

#### SUMMARY.

Alive, 102 . . . . . Injected, 47 (46 per cent). Controls, 55 (54 per cent).  
Dead, 58 . . . . . Injected, 33 (57 per cent). Controls, 25 (43 per cent).

tion of the table will show. It will be noticed that the percentage of injected animals left alive does not in many cases exactly correspond to the percentage of injected animals which were dead. This, however, is easily explained as due to the fact that the total number of individuals, both injected and controls, left alive was not equal to the total number of injected and controls which were dead. This irregularity would disappear with the use of a still larger number of animals in the experiments when the number left alive and the number dead would become more nearly equal.

Other small irregularities in the figures may also be noticed as,

for instance, the percentage left alive after the injection of 5 per cent alcohol (42 per cent) is greater than the percentage left alive after the injection of 4 per cent (40 per cent), when it should have been less. Also the percentage left alive after the injection of 2 per cent (47 per cent) is the same as after the injection of 1 per cent, when it should have been less. These irregularities

TABLE VIII.  
FUNDULUS INJECTED WITH 10 PER CENT ALCOHOL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min. 20 45	hrs. min. 2 30	4	6	4	6
20 45	2 30	4	6	6	4
18 00	2 30	5	5	4	6
17 00	2 30	8	2	9	1
17 45	2 30	8	2	6	4
17 45	2 30	8	2	10	0
Total . . . . .		37	23	39	21

SUMMARY.

Alive, 76 . . . . . Injected, 37 (48 per cent). Controls, 39 (52 per cent).  
Dead, 44 . . . . . Injected, 23 (53 per cent). Controls, 21 (47 per cent).

would undoubtedly disappear with the use of larger numbers of animals, and they do not in any way vitiate the general conclusion which may be drawn from the whole number of experiments; namely, that alcohol does not, in any strength, increase the resistance to lack of oxygen, but always causes a decrease, and in general this decrease is in proportion to the strength of the alcohol used.

Attention is to be called to the fact that, in general, *Fundulus* is very resistant to the ordinary effects of alcohol. At no time after the injection of alcohol in strengths up to 25 per cent were any symptoms of intoxication to be observed. An increased activity, loss of equilibrium, or narcosis were never noted. The

injected animals could not be seen to be any different from those used as controls.

With the injection of 30 per cent alcohol, however, effects began to be noticed. One or two in each experiment would show signs of narcosis, floating belly up at the surface of the water, with the respiratory movements much slower than normal. In most cases

TABLE IX.  
FUNDULUS INJECTED WITH 25 PER CENT ALCOHOL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
3 50	1 45	6	4	10	0
3 50	1 45	8	2	3	7
3 40	2 00	8	2	6	4
3 40	2 00	6	4	10	0
3 30	2 15	8	2	10	0
Total . . . . .		36	14	39	11

SUMMARY.

Alive, 75 . . . . . Injected, 36 (48 per cent). Controls, 39 (52 per cent).  
Dead, 25 . . . . . Injected, 14 (56 per cent). Controls, 11 (44 per cent).

recovery would take place within a few hours, when the animals would seem perfectly normal again. In a few cases, however, the animals would die. The abdomen in these cases was usually very red, showing an intense inflammation, brought about, no doubt, by the local irritant action of the alcohol on the peritoneum.

All these effects were more marked with the injection of 40 per cent alcohol. About five out of ten animals would be dead at the end of twenty-four hours after injection, with the same signs of inflammation in the abdomen. With the injection of 50 per cent, seven out of ten animals were dead, and with 60 per cent eight out of ten. Higher per cents of alcohol usually resulted in the death of all the animals, although occasionally one would survive the injection of even 80 per cent.

It has generally been recognized that alcohol when taken in

ordinary doses is very completely oxidized by the body, and that most of it disappears during the first ten or twelve hours. Atwater's<sup>17</sup> experiments indicate that fully 98 per cent is burned in the body, although the rate of oxidation is somewhat slower than had been ordinarily supposed, no evidence being found that it was burned any more rapidly than the ordinary nutrients of food.

In order to determine how quickly alcohol was oxidized by *Fundulus* a series of experiments were next undertaken to determine how quickly the effect of alcohol on resistance to lack of oxygen disappeared. In order to test this the fish were left several hours after injection before being placed under conditions of lack of oxygen; the definite length of time in each case is given in the tables. In the experiments given in Table VI from ten to fifteen minutes only had been allowed after injection before placing in the flask. Our results are shown in Tables VII-IX.

Table VII gives the results when between two and three hours' time after injection with 10 per cent alcohol was allowed before placing in the flask. The number of alive injected individuals, which was 37 per cent (Table VI) when only a few minutes elapsed between injection and placing in the flask, has now risen to 46 per cent. The number of dead injected animals, which was 59 per cent with the shorter time, has now decreased to 57 per cent. These results indicate that the effect of the injection of 10 per cent alcohol has decreased considerably by the end of two or three hours.

Table VIII gives the results when a still longer time (seventeen to twenty hours) was allowed between injection and placing in the flask. The number of alive injected individuals has now risen to 48 per cent, and the number of injected dead has fallen to 53 per cent, which is nearly within the limits of individual variation, and indicates that by the end of seventeen to twenty hours the effect of the alcohol had practically disappeared.

That a much shorter time even than that is needed before the effect disappears is shown by Table IX. The animals were here left not quite four hours between injecting with 25 per cent alcohol and placing in the flask. The number of alive injected animals, which was 27 per cent when only a few minutes had elapsed between injection and placing in the flask (Table VI), has

<sup>17</sup> ATWATER: *Loc. cit.*, ii, p. 243.

now risen to 48 per cent, and the number of injected dead individuals, which was 79 per cent with the shorter time, has now decreased to 56 per cent. Our results thus fall in line with the previous work in showing that during the first few hours alcohol is oxidized and disappears from the body.

In any discussion concerning the method of the action of alcohol in producing a decrease in the resistance to lack of oxygen there are several possibilities which must be considered.

1. A possible manner in which alcohol might decrease the resistance to lack of oxygen is suggested by some experiments on anaerobic respiration in the higher plants. It was in fact a consideration of these experiments which led in the first place to the testing of the effect of alcohol on resistance to lack of oxygen. Stoklassa and his pupils in various papers<sup>18</sup> have shown that in anaerobic respiration in seed plants, alcohol and carbon-dioxide are formed at the expense of the carbohydrates present and in the same proportion as in alcoholic fermentation by yeast. The conclusion is reached that the anaerobic metabolism of the seed plants is essentially identical with alcoholic fermentation by yeast. The first step is a conversion of sugar to lactic acid. The lactic acid is then split into alcohol and carbon-dioxide. Similar conclusions have also been reached by Palladin and Kostytschew,<sup>19</sup> who have found that the anaerobic respiration of etiolated bean and lupine leaves was very great after the addition of sugars, and also that the sugar-nourished leaves remained living a much longer time in lack of oxygen than leaves not so nourished. In these sugar-nourished leaves, in the absence of oxygen, alcohol and carbon-dioxide were found in the same proportion as in the fermentation of sugar by yeast, and therefore the anaerobic respiration might be considered to be a true alcoholic fermentation.

Now, in accordance with the law of chemical equilibrium, the rate of any chemical action decreases as the products of the action accumulate, and the action finally ceases when the products have attained a definite concentration. The accumulation of alcohol in a sugar solution undergoing fermentation with yeast gradually

<sup>18</sup> STOKLASSA: *Zeitschrift für die gesammte Biochemie*, 1903, iii, Heft 11. *Archiv für die gesammte Physiologie*, 1904, ci. *Berichte der deutschen botanischen Gesellschaft*, 1906, xxiv, p. 542.

<sup>19</sup> PALLADIN und KOSTYTSCHEW: *Berichte der deutschen botanischen Gesellschaft*, 1906, xxiv, p. 273; 1907, xxv, p. 51.

causes the fermentative processes to cease, and the addition of alcohol to the fermentating solution greatly decreases the growth of the yeast.<sup>20</sup> Hence, if alcohol is a product formed during respiration in the absence of oxygen, the accumulation of alcohol or the addition of alcohol to the tissues would decrease the amount of respiration in proportion to its concentration. This would shorten the length of time in which respiratory processes could go on in the absence of oxygen, and by that means decrease the resistance to lack of oxygen.

Alcohol has been found in minute quantities in the muscles of the higher animals.<sup>21</sup> It is probably a partial decomposition product of metabolism, but it is not known, so far as could be determined, whether it increases in quantity or not under conditions of lack of oxygen; so no positive comparison can be made between anærobic respiration in animals and in plants, nor can a definite statement be made as to whether or not alcohol acts in the manner stated above in decreasing the resistance to lack of oxygen.

2. In the second place it may be possible that the products of oxidation of the alcohol in the body may interfere with the processes of protoplasmic respiration. Alcohol on oxidation outside the body would form acetic acid, and while it is possible that the oxidation in the body may not give rise to the same products, yet Thomas<sup>22</sup> has shown that a volatile fatty acid is present in the blood of rabbits during intoxication, and the alkalinity of the blood was much reduced. The nature of this fatty acid was not determined, but since it is a fact that formic acid is excreted after methyl alcohol,<sup>23</sup> there can be but little doubt that the acid is acetic acid. This lowering of the alkalinity of the blood by the presence of the volatile fatty acid arising from the oxidation of the alcohol would greatly decrease the resistance of the animals to lack of oxygen.

It is well known that acids greatly retard protoplasmic oxidations, and the author has shown<sup>24</sup> that the injection of *m*/200 solu-

<sup>20</sup> HODGES: Influence of alcohol in growth; *Physiological aspects of liquor problem*, i, p. 361.

<sup>21</sup> RAJEWSKY: *Archiv für die gesammte Physiologie*, 1875, xi, p. 122.

<sup>22</sup> THOMAS: *Archiv für experimentelle Pathologie und Pharmakologie*, xli, pp. 3 and 4.

<sup>23</sup> POHL: *Archiv für experimentelle Pathologie und Pharmakologie*, xxxi, p. 281.

<sup>24</sup> PACKARD: *This journal*, 1905, xv, p. 30.

tion of acetic decreased the resistance of *Fundulus* to lack of oxygen. So it is possible that the effect of the alcohol may be due to its products of oxidation in the body.

3. In the third place the alcohol may be conceived of as affecting directly the respiration of the protoplasm, lowering its power of oxidation. There can be no doubt that while alcohol at times and in small quantities may serve to a limited extent as a food, yet it acts at all times as a drug and in large quantities is positively toxic, acting directly upon cell tissue. It is clearly a poison, retarding or even preventing metabolism, and as such would interfere with the respiration of the protoplasm.

The amount of alcohol necessary to produce a poisonous effect varies greatly with different living organisms. It can only be considered as a weak poison. It has been shown that algæ can withstand the effects of a 2 per cent solution for twenty-four hours, and infusoria tolerate a 1 per cent solution for some time.<sup>25</sup> Martin and Stevens<sup>26</sup> have shown, in the case of the isolated heart of a dog, that if the blood supplied to the heart contains one half of 1 per cent alcohol, the heart is no longer able to do its work. If the quantity of alcohol is less (*e. g.*, one fourth of 1 per cent), the effect on the heart is less, while if the percentage is only one eighth of 1 per cent, the alcohol has no influence at all.

Grehant<sup>27</sup> has shown that when the dog is in a profound state of intoxication the blood contains only one half of 1 per cent alcohol. The presence of less than 1 per cent alcohol in the blood was sufficient to cause death from respiratory paralysis. Carlson<sup>28</sup> states that the heart of *Limulus* will continue to beat for several hours with the ganglion in one fifth of 1 per cent alcohol, but with 1 per cent the primary stimulating phase is soon followed by depression and ultimate paralysis. The heart muscle itself is a little less sensitive.

In our own experiments with *Fundulus* the injection of 10 per cent alcohol, as given in the experiments in Table VII, is equivalent to 0.5 gm. of absolute alcohol per 100 gm. of body weight, or one half per cent of alcohol per body weight. The injection of 25 per cent alcohol in the experiments given in Table IX is equivalent

<sup>25</sup> Physiological aspects of liquor problem, ii, p. 13.

<sup>26</sup> MARTIN and STEVENS: Studies from Johns Hopkins Biological Laboratory, ii, p. 477.

<sup>27</sup> GREHANT: Journal de l'anatomie, xxxvi, p. 143.

<sup>28</sup> CARLSON: This journal, xvii, p. 177.



lent to 1.5 gm. of alcohol per 100 gm. of body weight, or 1½ per cent alcohol per body weight. These results show that fish are much more resistant to the toxic action of alcohol than is the dog, for the injection of 1.5 gm. of alcohol per 100 gm. of body weight was not sufficient to cause the death of the animals. Although the quantity above mentioned was not sufficient to cause death, it may have been great enough to have exerted a poisonous action upon the cell tissue sufficient to have decreased the resistance to lack of oxygen to the extent obtained in the experiments, and thus the action of alcohol in decreasing resistance to lack of oxygen may be explained on the basis of its toxic action upon protoplasm. When one considers the amount of alcohol which it is necessary to inject before any great decrease in resistance to lack of oxygen is obtained, it seems as if the view that alcohol acts as a weak poison must be the more probable explanation as to the manner in which alcohol acts in decreasing resistance to lack of oxygen.

#### V. EFFECT OF ACETONE ON RESISTANCE TO LACK OF OXYGEN.

Acetone was found by Palladin and Kostytschew<sup>29</sup> to be formed along with alcohol, under certain conditions, both in aerobic and anaerobic respiration of living and frozen plants. Acetone is also found in the urine in diabetes mellitus and fevers,<sup>30</sup> where it is supposed to be the result of an abnormal metabolism of the body's organized proteid. It is also found in the urine in other diseases in which there is an abundant destruction of body proteid.

As very little seemed to be known in regard to the pharmacological action of acetone, it was determined to test its effect on resistance to lack of oxygen. Acetone when treated with hydrogen is resolved into secondary propyl alcohol, and it was hoped that it might perhaps be able to act as a depolarizer, as the sugars do, and increase the resistance to lack of oxygen. Our results as summarized in Table X show that this is not the case, for when acetone was injected in solutions strong enough to produce any effect on resistance to lack of oxygen it always caused a decrease and never an increase. The injection of 0.1 per cent acetone had no effect upon the resistance to lack of oxygen, as an equal number of injected and controls were left alive and an equal number were

<sup>29</sup> PALLADIN und KOSTYTSCHEW: *Berichte der deutschen botanischen Gesellschaft*, 1906, xxiv, p. 273.

<sup>30</sup> JAKSCH: *Ueber Acetonurie und Diaceturia*, 1885.

dead. 0.5 per cent acetone caused a decrease in the resistance to lack of oxygen. Only 40 per cent of the injected were left alive, while 68 per cent of the injected were dead.

There is a still further decrease in the resistance to lack of oxygen with the injection of 5 per cent, though the effect is not so great as might be supposed from the sudden increase in the strength of the acetone used. Thirty-eight per cent of the injected were

TABLE X.  
FUNDULUS INJECTED ACETONE.<sup>1</sup>

Strength of acetone injected.	Alive.			Dead.		
	Total.	Injected.	Controls.	Total.	Injected.	Controls.
per cent.						
0.1	72	36 (50%)	36 (50%)	68	34 (50%)	34 (50%)
0.5	74	29 (40%)	45 (60%)	46	31 (68%)	15 (32%)
5.0	86	33 (38%)	53 (62%)	34	27 (79%)	7 (21%)

<sup>1</sup> In this table the summaries only of individual tables are used. With each per cent of acetone at least six experiments were made. In these experiments from ten to fifteen minutes were allowed for the absorption of the acetone after injection before placing in the flask.

left alive, and 79 per cent were dead. A series of control experiments were made to test any effect the acetone might have on the normal animals when they were not placed under conditions of lack of oxygen. The injection of 0.5 per cent acetone was followed by no effect that could be perceived. The animals seemed perfectly normal at all times. After the injection of 5 per cent acetone a few of the animals would show signs of narcosis within a few minutes, usually floating at the surface of the water, with the rate of respiratory movements much slower than normal. Recovery, however, took place after one to two hours, and the animals seemed perfectly normal again. The injection of 10 per cent acetone was followed by signs of narcosis in a greater number of animals, and usually at the end of twenty-four hours two or three individuals out of ten animals would be dead. It was therefore deemed useless to test the effect of this strength or any stronger solution on resistance to lack of oxygen.

Lack of time prevented the carrying out of experiments to determine how quickly the effect of the acetone disappeared. It may be judged, from the rather quick recovery that the animals made from the narcotic effects, that the acetone is oxidized and eliminated from the body more quickly than alcohol. Acetone produces narcosis more quickly and in weaker solutions than does alcohol, and its effects correspondingly more quickly disappear.

#### VI. THE EFFECT OF PILOCARPINE ON RESISTANCE TO LACK OF OXYGEN.

Mathews<sup>31</sup> has shown that the addition of small amounts of pilocarpine hydrochlorate to sea water (0.5 c.c. to 1 c.c. of one half per cent pilocarpine to 100 c.c. of sea water) hastens the development of embryos of starfish and sea-urchins and gives rise to abnormally large embryos. The explanation given is that the nature of the action suggests that pilocarpine increases the oxidations taking place in the cells. Sollmann<sup>32</sup> gives the same results. Pilocarpine added to sea water in concentration of 0.2 to 2.0 : 10,000 hastened the development of starfish to embryos. Larger doses produced the opposite effect.

If pilocarpine increases the oxidations of the protoplasm, there are two possibilities as to what its effect might be on resistance to lack of oxygen. The effect of the drug might be an increased power of oxidation, in which case the resistance to lack of oxygen would be increased. Or the effect might be an increased rapidity in the oxidative processes, in which case under condition of lack of oxygen the available supply of oxygen and oxidizable substances would be more quickly exhausted and the resistance to lack of oxygen would be decreased. The results of our experiments summarized in Table XI show that the latter is the case, for when pilocarpine was injected in solutions strong enough to have any effect on resistance to lack of oxygen it always caused a decrease and never an increase.

The injection of 0.01 per cent pilocarpine produced no effect on resistance to lack of oxygen, as exactly an equal number of injected animals (50 per cent) were left alive and were dead and a correspondingly equal number of controls were left alive and were dead. With the injection of 0.05 per cent the number of

<sup>31</sup> MATHEWS: This journal, 1901, vi, p. 207.

<sup>32</sup> SOLLMANN: This journal, 1904, x, p. 352.

alive injected animals has fallen to 27 per cent and the number of dead injected individuals has increased to 68 per cent. The effect is still more marked with the injection of 0.1 per cent. The number of alive injected animals is now only 23 per cent, while the number of dead injected is 72 per cent.

In control experiments where the animals were not placed under conditions of lack of oxygen but were left in running water for

TABLE XI.  
FUNDULUS INJECTED WITH PILOCARPINE.<sup>1</sup>

Strength of pilocarpine injected.	Alive.			Dead.		
	Total	Injected.	Controls.	Total.	Injected.	Controls.
per cent.						
0.01	106	53 (50%)	53 (50%)	94	47 (50%)	47 (50%)
0.05	52	14 (27%)	38 (73%)	68	46 (68%)	22 (32%)
0.1	62	14 (23%)	48 (77%)	78	56 (72%)	22 (28%)

<sup>1</sup> In this table the summaries only of individual tables are given. With each per cent of pilocarpine at least eight experiments were made. From ten to fifteen minutes were allowed for absorption of the pilocarpine after injection before placing in the flask.

many hours after injection, the injection of 0.05 per cent pilocarpine produced no visible effect on the animals. Their activities, so far as could be seen, seemed perfectly normal. The injection of 0.1 per cent would generally cause the death of about one animal out of ten at the end of twenty-four hours. This effect was still greater with the injection of 0.5 per cent, which would cause the death of about three animals out of every ten. Doses of this strength were therefore poisonous, and could not be used in testing the effect on resistance to lack of oxygen.

No experiments were made to determine how long the effect of pilocarpine in decreasing resistance of lack of oxygen would last.

#### SUMMARY.

1. Mannose, when injected intraperitoneally into *Fundulus heteroclitis*, increases their resistance to lack of oxygen. Its effect is

the same as that found in previous work for maltose, glucose, and levulose, and the same explanation of its action is given; namely, that it acts as a depolarizer in the processes of protoplasmic respiration. Galactose is apparently not absorbed from the body cavity and so cannot increase the resistance to lack of oxygen.

2. Linseed oil and olive oil when fed into the stomach by means of a pipette have no effect on resistance to lack of oxygen. It could not be determined whether the fats were absorbed or not. Linseed oil when injected intraperitoneally causes a decrease in the resistance to lack of oxygen. The oil was probably not absorbed from the body cavity, and no explanation of this effect is suggested. No importance is attached to this result from the standpoint of respiration.

3. Ethyl alcohol in concentration of 40 per cent and upward produces death in some of the animals injected. In all strengths below this it causes a uniform decrease in resistance to lack of oxygen. The decrease is slight with 1 per cent, but gradually grows greater in proportion as the strength of alcohol used is increased. The effect of the alcohol is probably due to its toxic action on the protoplasm, lowering its powers of respiration. The alcohol is oxidized within four or five hours after injection, and the effect on resistance to lack of oxygen disappears.

4. Acetone causes a decrease in resistance to lack of oxygen in all strengths from 0.5 per cent up to that which is poisonous and produces death (5.0 per cent). Weaker solutions have no effect. Acetone is thus more toxic than alcohol, but probably acts in the same manner as alcohol.

5. Pilocarpine in strengths from 0.05 per cent to 0.1 per cent causes a decrease in resistance to lack of oxygen in proportion to the concentration used. Weaker solutions have no effect, and stronger solutions are poisonous. Since pilocarpine is supposed to increase the oxidations of the protoplasm, this effect is probably due to the more rapid use of the available oxidizable substances, which shortens the length of life in lack of oxygen.

I wish to thank the Director and Assistant Director of the Marine Biological Laboratory for the privilege of occupying a research room. My thanks are also due to Prof. A. P. Mathews for assistance and direction.

## THE DIRECT UTILIZATION OF THE COMMON SUGARS BY THE TISSUES.

By HUGH MCGUIGAN.

[From the Laboratory of Physiology and Pharmacology, Washington University, St. Louis, Missouri.]

THE form in which carbohydrates must reach the tissues to be available as food is not definitely known. Many believe that dextrose is used directly as such. Others think that it must be polymerized and reach the tissues as glycogen or in some other colloidal form before it is oxidized.<sup>1</sup> The mechanism of the oxidation is *a priori* little understood. Regarding the fate of levulose much more uncertainty prevails; by many it is held that the levulose is converted into glycogen, and this again into dextrose, before oxidation into its final products. Lobry de Bruyn and Van Eckenstein<sup>2</sup> have shown that such a transformation is possible in vitro. It is well known that under some conditions these sugars act very differently in the economy. It is also known that the ease with which they are oxidized is different.<sup>3</sup> The sugar of the blood exists normally in colloidal combination.<sup>4</sup> This is taken by some to mean that the colloidal form only is used in the economy. The present research is an attempt to determine the forms in which sugar can be utilized by the tissues.

<sup>1</sup> VAN NOORDEN: Diabetes, New York, 1905, p. 57. See, also, LUSK: Science of nutrition, p. 240; PAVY: The carbohydrates, London, 1906.

<sup>2</sup> LOBRY DE BRUYN and ALBERDA VAN ECKENSTEIN: Berichte der deutschen chemischen Gesellschaft, 1895, xxviii, p. 3078; LOBRY DE BRUYN and VAN LUNT: *Ibid.*, 1907, xxix, p. 594; Bulletin de la Société de Chimie de Paris, (3) xv; Chemisches Centralblatt, 1896, ii, and 1897, ii.

<sup>3</sup> BUNZEL, H. H.: This journal, 1908, xxi, p. 23; MCGUIGAN, H.: *Ibid.*, 1907, xix, p. 175; MATHEWS, A. P.: *Ibid.*, 1907, xix, p. 199.

<sup>4</sup> LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 410; PAVY: The carbohydrates, 1905, p. 69; STILES and LUSK: Science of nutrition (Lusk), 1906, p. 228; MCGUIGAN and BROOKS: This journal, 1907, xviii, p. 256.

METHOD.

The method of investigation consisted essentially of perfusing organs with the sugars under consideration.<sup>5</sup> Animals were bled to death under ether anaesthesia; the defibrinated blood was filtered through cheese cloth and diluted with Ringer-Locke's solution and kept at 37°-40° C. during the perfusion. A known quantity of sugar was added to this solution, and the amount of sugar in the whole determined at once by Pavy's method. The organ was perfused through the artery with the solution at a temperature of 37°-40° and collected from the vein. The amount of the loss of sugar could thus be determined. Details and explanations are given in the protocols.

**December 11, 1907.** — Perfusion of cat's hind legs with dextrose solution.

3.10. Cat bled, 90 c.c. blood diluted to 400 c.c. with Ringer-Locke's solution and sugar added. Sugar content 3.1916 per thousand.

3.20. Perfusion commenced. 37° C.

3.25. Electrical stimulation of muscles, 25 per minute, gave double contractions. The blood leaving the veins was very dark in color and was oxygenated by shaking with the air between each perfusion.

3.30. 125 c.c. ran from the vein in ten minutes.

3.37. First perfusion ended. Sample II taken for analysis.

3.50. Second perfusion ended.

4.05. Third perfusion ended.

4.29. Fourth perfusion ended.

4.35. Fifth perfusion ended. Blood squeezed from tissues by hand.

*Summary of results.* —

Blood before perfusion contained 3.1916 sugar per litre

Blood after first perfusion (20 min. from beginning) 2.9659 sugar

Blood after second perfusion (30 min. from beginning) 2.7750 sugar

Blood after third perfusion (45 min. from beginning) 2.4736 sugar

Blood after fourth perfusion (65 min. from beginning) 1.9473 sugar

Blood after fifth perfusion (71 min. from beginning) 1.5384 sugar

<sup>5</sup> The apparatus used was that designed by GUTHRIE and PIKE and described in this journal, 1907, xviii, p. 15.



It is readily seen that a large reduction of sugar has taken place. This loss, however, could be accounted for in one of several ways: (1) by actual oxidation; or (2) by leakage into the surrounding tissues; or (3) by storage as glycogen or other forms in the tissues. Examination shows that there is an actual combustion of the sugar, that there is no glycogen formation, and that while transudation occurs it accounts for only a part of the loss.

**December 12, 1907.** — Experiment to determine whether the liver uses sugar in work. A medium-sized cat was taken. The liver was perfused through the portal vein. One lobe was tied off at the beginning of the experiment to be analyzed for glycogen and sugar. Another part was taken at the end of the experiment for the same purpose.

1.50. Cat bled, 42 c.c. blood to which 6 c.c. 10 per cent glucose was added, and the volume made to 200 c.c. with Ringer-Locke's solution.

2.00. Perfusion started. Sample I taken.

2.10. First perfusion ended. Sample II taken.

3.00. After perfusing many times Sample III was taken.

The blood leaving the veins was very dark in color. The perfusion pressure was 22 cm. of blood.

*Results of analysis of perfused blood.* —

Sample I . . . . .	8.2058 per mille sugar
Sample II . . . . .	7.0961 per mille sugar
Sample III . . . . .	6.7796 per mille sugar

A marked destruction of sugar is apparent. Unfortunately one of the flasks containing the liver samples for glycogen was accidentally broken, and it was impossible to determine whether or not there was a glycogen storage. However, a second experiment carried out in the same manner for the determination of glycogen and sugar in the liver showed no storage of glycogen under the conditions of the experiment. By a more rapid restoration of the circulation a storage of glycogen occurs in the liver.<sup>6</sup>

**December 13, 1907.** — Utilization of sugar by the liver.

3.24. Cat anesthetized and bled 39 c.c. This was mixed with 50 c.c. from another cat and defibrinated. 10 c.c. 10 per cent dextrose was added and the whole made to 600 c.c. with Ringer-Locke's solution and analyzed.

<sup>6</sup> GRUBE: *Journal of physiology*, 1903, xxix, p. 276.

- 3.47. Commenced perfusion through both portal vein and hepatic artery.
- 3.50. Ligated lobe 1 of liver. Sample I taken.
- 3.53. 84 c.c. blood perfused in eight minutes.
- 3.59. Sample II taken.
- 4.10. Perfusion rate 53 c.c. in two minutes. The same blood (100 c.c.) being used repeatedly.
- 4.25. Sample III taken. Perfusion rate 60 c.c. in two minutes.
- 4.27. Sample IV taken. Hepatic artery shut off.
- 4.30. Sample V taken. Perfusion rate 56 c.c. in three minutes.

*Results of analysis. —*

Blood at beginning . . . . .	8.9000 per mille
Sample II after 9 minutes . . . . .	8.1200 per mille
Sample III after 35 minutes . . . . .	9.1000 per mille
Sample IV after 37 minutes . . . . .	6.6400 per mille
Sample V after 40 minutes . . . . .	2.5600 per mille
Lobe 1 at beginning (glycogen and sugar) . . . . .	1.0154 per cent
Lobe 2 at end (glycogen and sugar) . . . . .	0.4325 per cent

Instead of a storing, a loss of glycogen took place.

**December 17, 1907.** — Experiment to show that the liver uses sugar in work. Cat's liver perfused with oxygenated cat's blood.

- 1.25. Cat bled 65 c.c. Diluted with Locke's solution; sugar added and volume made to 260 c.c.
- 1.34. Perfusion started through portal vein. Lobe 1 tied off for control. Sample I for sugar analysis taken, 25 c.c.
- 1.42. Perfusion rate 100 c.c. in six minutes.
- 1.50. Perfusion rate 95 c.c. in eight minutes.
- 1.57. Second perfusion ended.
- 2.04. Sample II taken, lobe 2 of liver tied off.
- 2.36. Sample III taken.
- 2.59. Sample IV taken, 125 c.c. remained.

Total weight of liver . . . . .	65.00 gm.
Weight of lobe 1 . . . . .	7.50 gm.
Weight of lobe 2 . . . . .	6.50 gm.
Weight of lobe 3 . . . . .	25.50 gm.

*Results of analysis. —*

	Per cent.
Blood at beginning of experiment . . . . .	0.7680
Sample II after perfusing for 30 minutes . . . . .	0.6440
Sample III after perfusing for 58 minutes . . . . .	0.6180
Sample IV after perfusing for 85 minutes . . . . .	0.6520

	Per cent.
Glycogen as sugar, lobe 1 . . . . .	0.8666
Glycogen as sugar, lobe 2 . . . . .	0.7692
Glycogen as sugar, lobe 3 . . . . .	0.8520
Sugar as such, lobe 1 . . . . .	0.4666
Sugar as such, lobe 2 . . . . .	0.4230
Sugar as such, lobe 3 . . . . .	0.2640
Total carbohydrates, lobe 1 . . . . .	1.3332
Total carbohydrates, lobe 2 . . . . .	1.1922
Total carbohydrates, lobe 3 . . . . .	1.1160

No glycogen was stored. In fact some was lost, but there was a constant loss of sugar, which shows that the sugar-destroying power persists for some time after the glycogen-storing function is lost. Under proper precautions a glycogen-storing power can be demonstrated by perfusion.<sup>6</sup> Embden<sup>7</sup> states that if a glycogen-free liver be perfused with normal blood, an increase in sugar may take place. A probable explanation for this is given by Lusk.<sup>8</sup> He thinks that such substances as lactic acid, glycolaldehyde, etc., may account for the glycogen formation. Such results cannot be expected in the isolated liver. For a time the blood coming from the liver may show an increase in sugar, due to hydrolysis of the liver glycogen.

**The utilization of dextrose by the muscles. December 19, 1907.**—Perfusion of hind legs of rabbit. Weight 2400 gm.

11.15. Killed by blow on head and blood collected, 50 c.c.; this was made to 400 c.c. with Ringer-Locke's solution plus 6 c.c. 10 per cent dextrose.

11.29. Sample I, 25 c.c. taken for analysis. Sample II, 25 c.c. set aside at 40° to determine glycolysis.

11.30. Perfusion commenced and legs stimulated 30 times per minute.

11.48. First perfusion ended. Sample III taken.

12.07. Second perfusion ended.

12.08. Sample IV taken.

12.18. Third perfusion ended. Sample V taken.

12.32. Fourth perfusion ended. Sample VI taken.

Time of perfusion, one hour and two minutes.

<sup>6</sup> GRUBE : *Journal of physiology*, 1903, xxix, p. 276.

<sup>7</sup> EMBDEN : *Hofmeisters Beiträge*, 1904, vi, p. 44.

<sup>8</sup> LUSK : *Loc. cit.*, p. 232.

*Results.*—

Blood used for samples . . . . .	150 c.c.
Amount recovered at end of experiment . . . . .	110 c.c.
Amount lost in perfusion by transudation and collateral circulation . . . . .	140 c.c.
Sample I sugar content at beginning . . . . .	0.1790%
Sample II sugar content at 40° for 1 hour (glycolysis, 0.0136 per cent) . . . . .	0.1654%
Sample III sugar content after perfusing 18 minutes . . . . .	0.1616%
Sample IV sugar content after perfusing 44 minutes . . . . .	0.1552%
Sample V sugar content after perfusing 62 minutes . . . . .	0.1480%

The perfused legs were left after the perfusion in the open room until 1.50 P. M. (one hour and thirty minutes) and again perfused. By this time there was no reaction to stimulation. 110 c.c. of the blood left from the previous experiment was made to 300 c.c. with Ringer-Locke's solution and 5 c.c. 10 per cent (approximately) dextrose solution added.

- 1.50. Sample I, 25 c.c. taken for analysis.
- 1.52. Perfusion commenced.
- 2.15. Blood perfused twice. Sample II taken.
- 2.35. Blood again perfused twice. Sample IV taken.
- 2.50. Blood again perfused twice. Sample V taken.

*Results of analysis.*—

	Per cent.
Sample I sugar content . . . . .	0.2340
Sample II sugar content . . . . .	0.2280
Sample III sugar content . . . . .	0.2060
Sample IV sugar content . . . . .	0.1800
Sample V sugar content . . . . .	lost

In this case the leg was inactive to stimulation. The cells were either dying or were already dead. Yet there was a sugar loss. It could not have been stored as glycogen in this case, and either (1) the slowly dying cells use sugar after they are unable to respond to stimulation, or (2) there was a transudation of sugar into the tissues, or (3) the mere perfusion through such a mechanical device may oxidize the sugar. Before we can say that the tissues use sugar directly, these questions must be answered.

**December 19, 1907.**—Second experiment. Dead leg perfused with dextrose showed practically no loss of sugar, but a marked transu-

dition of the sugar into the tissues resulted. The experiment was repeated with galactose with practically the same results. The figures obtained are given under the discussion of that sugar.

**December 26, 1907.**—The legs of the dog were perfused as before, except that the legs were cut from the body.

10.18. Dog bled 220 c.c., made to 400 c.c. with Ringer-Locke's solution and 4 c.c. 10 per cent sugar solution added.

10.40. Sample I taken for immediate analysis, and Sample II set aside for glycolysis.

10.45. Perfusion commenced.

10.54. Stimulation 32 per minute gave strong response.

11.12. First perfusion ended. 300 c.c. ran through in thirty-seven minutes.

11.42. Second perfusion ended.

12.07. Third perfusion ended.

12.35. Fourth perfusion ended.

1.02. Fifth perfusion ended.

Time, two hours and seventeen minutes. The leg still responded when stimulated.

*Results.*—

	Per cent.	Gm.
Blood at beginning of perfusion 400 c.c. . . . .	0.1510	0.6040
Blood at end of perfusion 266 c.c. . . . .	0.1090	0.2899
Sugar in non-perfused leg . . . . .	0.1588	
Glycogen in non-perfused leg . . . . .	0.08	
Weight of non-perfused leg 245 gm.		
Total carbohydrate as dextrose in non-perfused leg . . . . .	0.2388	0.5850
Sugar in perfused leg . . . . .	0.1775	
Glycogen in perfused leg . . . . .	0.08	
Weight of leg 262 gm. after perfusion		
Gain of sugar in leg by perfusion . . . . .		0.0896
Total carbohydrate as dextrose in leg after perfusion . . . . .	0.2575	0.6746
Sugar recovered from Sample I . . . . .	0.1510	0.0375
Sugar recovered from Sample II . . . . .	0.1350	0.0338
Sugar in blood at end (226 c.c.) . . . . .	0.1090	0.2899
Total sugar accounted for at end . . . . .		1.0358
Total sugar at beginning . . . . .		1.1890
Loss in perfusion . . . . .		0.1432
Loss that could have occurred by glycolysis alone in the 350 c.c. blood perfused . . . . .	0.016	0.0560

When everything is taken into consideration, there is still a loss of sugar. A leg weighing 262 gm. in this case used 0.0872 gm. dextrose in two hours when the concentration of the sugar in the blood was about normal (0.1510 per cent); with a stronger concentration a greater amount of sugar is oxidized.

**January 19, 1908.** — The utilization of levulose.

10.00. A young dog, weight 12 kilos, was bled to death as in the previous experiments. 500 c.c. defibrinated blood was made to 800 c.c. with Ringer-Locke's solution with the addition of 90 c.c. 5 per cent levulose (approximately).

10.30. Perfusion of leg started. Sample I, 25 c.c. taken for sugar determination. Sample II, 25 c.c. set aside for glycolysis at 40° C. during the perfusion.

10.55. First perfusion ended, 400 c.c. Sample III taken.

11.15. Stimulation 30 per minute. Gave double contractions.

11.40. Second perfusion ended and Sample IV taken.

12.35. Third perfusion ended. Sample V taken.

1.00. Fourth perfusion ended. Sample VI taken after blood was squeezed from leg.

Weight of perfused leg . . . . . 1000 gm.

Weight of non-perfused leg . . . . . 1050 gm.

Weight of muscle of perfused leg . . . . 610 gm.

Weight of muscle of non-perfused leg . . 620 gm.

100 gm. of muscle from each leg was taken for glycogen and sugar determination.

VIII. 100 gm. perfused leg for sugar.

IX. 100 gm. perfused leg for glycogen.

X. 100 gm. non-perfused leg for sugar.

XI. 100 gm. non-perfused leg for glycogen.

*Results of analysis.* —

	Per cent.
I. Sugar at beginning . . . . .	0.5472
II. Sugar after glycolysis at 40° C. during the perfusion .	0.5432
III. Sugar after first perfusion (25 minutes) . . . . .	0.5068
IV. Sugar after second perfusion (50 minutes) . . . . .	0.4628
V. Sugar after third perfusion (2 hours, 5 minutes) . . .	0.4348
VI. Sugar after fourth perfusion (2 hours, 30 minutes) . .	0.4348

In the last case the blood was squeezed from the tissues.

	Gm.
Sugar in non-perfused leg, 0.12 per cent, 1050 gm. .	1.260
Sugar in perfused leg, 0.1645 per cent, 1000 gm. .	1.645

	Gm.
Gain in perfused leg . . . . .	0.4045
Glycogen, no difference in legs, 0.08 per cent in each.	
Sugar in blood 500 c.c. at beginning, 0.5432 per cent . . .	2.7160
Sugar in blood 310 c.c. at end, 0.4348 per cent . . .	1.3478
Total loss of sugar from blood . . . . .	1.3682
Sugar recovered from Samples III, IV, and V . . .	0.3508
Difference in carbohydrate content of muscles at end .	0.3850
Total sugar loss . . . . .	0.6324

The amount of dextrose in the original blood was not determined, but if this be calculated at one part per thousand, there would be 312 c.c., or 0.3120 gm. dextrose. And if we admit that the dextrose is used first, which is improbable, it would still leave 0.3204 gm. of the levulose used. It is true there is a loss of 115 c.c. of blood in the experiment, which, if added to the above, would account for the loss of the sugar in the legs before and after perfusion, which again would leave some sugar unaccounted for and which must have been used in muscular work. The actual gain of weight by transudation was not determined in this experiment.

**January 21, 1908.** — The utilization of levulose.

Dog forty-eight hours without food was anesthetized as before, bled from carotid 250 c.c., made to 380 c.c. with Ringer-Locke's solution with levulose added and used in perfusion. Samples of the blood I, and after the addition of levulose II, were taken for analysis.

9.57. Perfusion of leg commenced. Weight of leg before perfusion 670 gm.

10.02. Second perfusion ended. Sample III taken for immediate analysis. Sample IV set aside at 40° for glycolysis.

10.15. Second perfusion ended, 275 c.c.

10.30. Third perfusion ended.

10.40. Fourth perfusion ended. Sample V taken.

11.07. Seventh perfusion ended. Sample VI taken.

11.38. Ninth perfusion ended. Sample VII taken.

12.18. Twelfth perfusion ended. Sample VIII taken. Reaction to stimulation decreasing but still vigorous.

12.48. Fifteenth perfusion ended. Sample IX taken.

1.10. Sixteenth and final perfusion ended. Sample X taken. Leg still reacts to stimulation. Weight of leg 701 gm. Weight of muscles 400 gm. Samples of 50 gm. of muscle were taken for sugar and glycogen analysis.



Amount of blood at beginning . . . . .	380 c.c.
Amount taken as samples . . . . .	225 c.c.
Amount left at end of experiment . . . . .	75 c.c.
Amount unaccounted for . . . . .	80 c.c.
Gain in weight of leg . . . . .	31 gm.

A very small amount of blood remained in the apparatus, and a slight amount was lost in work.

*Results.* —

Dextrose in blood before dilution . . . . .	0.1250%
Dextrose in perfused blood . . . . .	0.0801%
Total sugar in perfused blood calculated as dextrose . . . . .	0.8620%
Sugar in blood at end of perfusion . . . . .	0.3360%
Weight of muscles in leg . . . . .	400 gm.
Sugar content before perfusion . . . . .	0.0300% 0.1200 gm.
Sugar content after perfusion . . . . .	0.1500% 0.6000 gm.
Gain in sugar by perfusion . . . . .	0.4800 gm.
Glycogen in muscles before perfusion . . . . .	0.0750% 0.3000 gm.
Glycogen in muscles after perfusion . . . . .	0.0660% 0.2640 gm.
Loss in glycogen during perfusion . . . . .	0.0360 gm.
Total gain of carbohydrate in leg . . . . .	0.4440 gm.
Sugar at beginning of experiment, 390 c.c. . . . .	0.8620% 3.2756 gm.
Sugar in recovered blood, Samples III-X . . . . .	1.3060 gm.
Sugar in 75 c.c. left at end . . . . .	0.5260% 0.3945 gm.
Loss of sugar from blood . . . . .	1.5751 gm.
Total carbohydrate gain in leg . . . . .	0.4440 gm.
Total loss of sugar as dextrose . . . . .	1.1311 gm.
Dextrose in blood at beginning . . . . .	0.0801% = 0.3124 gm.
Levulose loss not less than . . . . .	1.1311 — 3.124 = 0.8187 gm.

There is a distinct loss of levulose here of not less than 0.8187 gm. which can only be accounted for by oxidation in the muscles. The low per cent of glycogen in the muscles is due to the glycogen's being hydrolyzed. No attempt was made to determine the amount of each as they existed when the animal was bled, but usually an hour or more intervened between the death of the animal and the taking of the muscle samples for glycogen and sugar.

**January 23, 1908. Maltose.** — The action of the tissues on maltose was determined in the same manner as in glucose and levulose. A young dog was bled to death as above. The amount of blood was 295 c.c. Sample I was taken for the determination of glucose.

The remaining 270 c.c. was made to 400 c.c. with Ringer-Locke's solution, to which maltose was added. The weight of the leg perfused was 653 gm. 400 c.c. of solution was used in the perfusion.

- 10.20. Sample II taken and perfusion commenced.
- 10.30. Stimulation 30 per minute.
- 10.50. First perfusion ended. Sample III taken.
- 11.12. Second perfusion ended.
- 11.15. Stimulation.
- 11.28. Third perfusion ended.
- 11.35. Stimulation stopped. Leg still reactive.
- 12.05. Stimulation applied again.
- 12.08. Fourth perfusion ended.
- 12.38. Fifth perfusion ended and Sample IV taken.
- 1.08. Seventh perfusion ended.
- 1.30. Eighth perfusion ended.
- 3.00. Fourteenth and last perfusion ended. Blood at end 265 c.c. Sample V taken for analysis.

The leg was stimulated about five minutes, then allowed to rest five minutes alternately during the experiment. The reaction at the end was very weak, but still distinct.

*Results. —*

Weight of leg at end of experiment . . . .	682 gm.
Weight of leg at beginning of experiment . . .	653 gm.
Gain in weight by transudation . . . .	27 gm.
Weight of muscle from perfused leg . . . .	234 gm.
Amount taken for sugar determination . . .	50 gm.
Amount taken for glycogen determination . .	50 gm.

*Results of analysis. —*

I. Sugar in original blood as dextrose . . . . .	0.1500%
II. Sugar in blood after addition of maltose . . . . .	0.6325%
III. Sugar in blood at end of first perfusion (30 min.) . .	0.5720%
IV. Sugar in blood at end of fifth perfusion (2 hrs. 8 min.)	0.5720%
V. Sugar in blood at end of experiment (4 hrs. 16 min.)	0.4720%
Sugar in non-perfused leg . . . . .	0.0500%
Sugar in perfused leg . . . . .	0.2250%
Total sugar in blood at beginning of experiment . .	2.5300 gm.
Recovered from Samples II-V . . . . .	0.5622 gm.
Recovered from blood at end of experiment . . . .	1.1328 gm.
Difference in muscles . . . . .	0.4587 gm.
Total loss . . . . .	0.3763 gm.
Total dextrose in perfused blood (253 c.c. original) .	0.3795 gm.

The total quantity of sugar as dextrose that could have been oxidized here is 0.3763 gm. If we assume that the dextrose alone was oxidized, then the maltose remained unoxidized. A noticeable feature of the results with maltose is that perfusion between the period of thirty minutes and two hours and eight minutes (III and IV in the table) gave no loss of sugar. Then between this period and the end of the experiment there was a large reduction. I take this to mean that transudation of the sugar does not take place while the muscles and vessels are living and in good condition, but immediately on losing their tone, or when they are dying, transudation takes place. The sugar destruction probably takes place in the first part of the perfusion and the transudation into the tissues marks a later stage.

**The utilization of galactose.** — The legs of a young dog were perfused with blood containing a known quantity of galactose. The weight of the perfused leg at the beginning of the experiment was 418 gm. The blood of the animal contained 0.1750 per cent dextrose; 300 c.c. of this was diluted to 600 c.c. with Ringer-Locke's solution containing galactose. The perfused blood contained 0.7290 per cent sugar, of which 0.0875 per cent was the blood sugar; the remainder was galactose. The perfusion lasted two hours and twenty minutes. During this time the blood was perfused six times. The following brief summary of the experiment will explain:

- 10.30. Animal bled 325 c.c. blood.
- 10.45. Perfusion commenced. Stimulation 30 per minute. Reaction vigorous.
- 11.10. First perfusion ended.
- 11.35. Second perfusion ended. Reaction to stimulation vigorous.
- 11.35. Third perfusion ended. Reaction to stimulation weak.
- 12.00. Fourth sample of blood taken for analysis.
- 12.20. Fourth perfusion ended. No response to stimulation.
- 12.40. Fifth perfusion ended. Leg becoming edematous.
- 1.05. Sixth perfusion ended.

The following samples were taken for analysis:

- I. Original blood of animal.
- II. Blood after addition of galactose.
- III. Blood which had been set aside at 40° C. during the experiment.

The sugar content of these samples was found to be:

	Per cent.
I. Sugar content of normal blood . . . . .	0.1750
II. Sugar content after the addition of galactose . . . . .	0.7290
III. Sugar content after standing 2 hrs. 20 min. at 40° C. . . . .	0.7260
IV. Sugar content after 1 hr. 15 min. perfusion through living leg . . . . .	0.6320
V. Sugar content after 2 hrs. 20 min. perfusion . . . . .	0.5400
A. Sugar in non-perfused leg muscle . . . . .	0.1950
B. Sugar in perfused leg muscle . . . . .	0.3320

In the last two samples, A and B, the greater part of the glycogen in the leg was in all probability converted into dextrose.

Weight of leg before perfusion . . . . .	418 gm.
Weight of leg after perfusion . . . . .	493 gm.
Gain in weight by perfusion . . . . .	75 gm.
Weight of bone, skin, etc. . . . .	238 gm.

A summary of these results will show the amount of glycolysis:

	Gm.
Sugar at beginning of experiment in blood . . . . .	3.9930
Sugar at beginning of experiment in leg muscles . . . . .	0.3315
Total . . . . .	4.3245
Sugar recovered in blood at end of experiment . . . . .	2.2950
Sugar in Sample IV . . . . .	0.1580
Sugar in muscle at end, 0.3320 per cent . . . . .	0.5644
Total sugar recovered . . . . .	3.0134
Sugar loss . . . . .	1.3111
Total dextrose in blood at beginning . . . . .	0.5270
Galactose loss not less than . . . . .	0.7841

The perfusion of the dead leg is more difficult, and the results are not so satisfactory for several reasons. The principal difficulty is the edematous condition that begins with the perfusion. There is no difficulty in circulating the blood through the vessels, but the gradually increasing edema and increasing viscosity of the blood caused by the transudation of the water and salts into the tissues makes it necessary to increase the pressure required to keep the perfusion nearly at the same rate. Edema takes place in all parts of the legs and even in the feet. The subcutaneous tissue is also saturated. This condition renders difficult the exact determination of the sugar in the muscles after the perfusion, and for exact work

the whole leg should be used. The blood leaving the veins in case of the dead legs never shows any of the characteristics of venous blood, but is as arterial in color as before the perfusion.

A summary of the results of perfusion of the dead leg with galactose is given:

	Gm.
Blood at commencement of perfusion, 360 c.c. at 0.9320% = . . . . .	3.3550
Sugar in muscles of same weight taken from fore-legs . . . . .	0.4575
Total sugar at beginning . . . . .	3.8125
Sugar recovered in blood (200 c.c.) . . . . .	1.3680
Sugar recovered in muscle . . . . .	2.1956
Total sugar recovered . . . . .	3.5636
Total sugar unrecovered . . . . .	0.2389 or 6%

This loss is due, I think, entirely to edema into the skin, and non-muscular structures, which could not be recovered by the mere analysis of the muscular tissues. Had the whole leg been ground and used in the analysis, the recovered sugar would undoubtedly have approached the original more closely. The difference given does not vitiate the conclusion that the sugars are used directly by the living cell and not by dead tissues.

The results of these experiments show that the common sugars, dextrose, levulose, and galactose, are used directly by the cells. Maltose, in all probability, is not directly oxidized, or, at most, only a minute amount.

Since commencing this work, a paper by Locke and Rosenheim,<sup>9</sup> "On the consumption of dextrose by mammalian cardiac muscle," has reached me. These authors find that the contracting heart muscle uses dextrose. I fully agree with their results. In fact I think they would have found a greater utilization of sugar had they used blood for perfusion. The best artificial blood substitutes are poor oxygen carriers. I find the same thing to hold true for muscular tissues. The contracting muscular tissues, like the contracting heart muscle, utilizes dextrose directly. From the results of Kulz<sup>10</sup> and Jensen<sup>11</sup> it would seem that glycogen is not directly

<sup>9</sup> LOCKE and ROSENHEIM: *Journal of physiology*, 1907, xxxvi, p. 205.

<sup>10</sup> KULZ: *Festschrift für Ludwig*, 1891, p. 109; quoted from LUSK, *Loc. cit.*, p. 149.

<sup>11</sup> JENSEN: *Zeitschrift für physiologische Chemie*, 1902, xxxv, p. 525.

used by the heart. Levulose and galactose are directly utilizable. The statement will in all probability hold true for all the directly fermentable sugars. Chauveau and Kaufmann<sup>12</sup> found that the muscles in action used 3.5 times as much sugar as the inactive living muscle. Results on stimulating the excised legs of dogs indicate in general that these results are in the right direction, but we have not made quantitative determinations. Mere analysis of the blood going to and from the muscle is not sufficient to show the amount of the sugar used, as there may be a transudation of the sugar into the surrounding tissues. However, with normal blood in the intact animal, this objection may have no weight. I do not believe, however, that the amount of sugar utilized by the intact body can be determined by analysis of blood and urine as attempted by Pavy.<sup>13</sup> There is always a transudation of sugar from the vessels which must be considered. On increasing the sugar content of the blood this transudation may occur in any part of the body, but greatest in quantity and most easily in the normal kidney.

**Products of sugar oxidation.** — Von Frey and Gruber<sup>14</sup> perfused blood through the hind legs of dogs, and tetanized the muscles and determined the lactic acid content before and after the perfusion. In one case they found the lactic acid increased ten times. Berlinerblau<sup>15</sup> confirmed this result, but observed also that when sugar was added to the perfused blood the formation of lactic acid was increased. Oppenheimer<sup>16</sup> observed the same phenomena with drawn blood at 37° C. I have found that the amount of sugar utilized was increased if the content in the blood was increased, but have not yet determined the product of glycolysis or the lactic acid<sup>17</sup> content. Other experimenters have reported an increase in lactic acid. On the other hand, Asher and Jackson,<sup>18</sup> by transfusing blood with and without the addition of sugar through the hind legs of

<sup>12</sup> CHAUVEAU and KAUFMANN, *Comptes rendus de l'Académie des Sciences*, 1887, civ, pp. 1126, 1352, and 1409.

<sup>13</sup> PAVY: *Journal of physiology*, 1890, xxiv, p. 479.

<sup>14</sup> VON FREY: *Archiv für Physiologie*, 1885, p. 533; VON FREY and GRUBER: *Ibid.*, 1885, p. 519.

<sup>15</sup> BERLINERBLAU: *Archiv für experimentelle Pathologie und Pharmakologie*, 1887, xxiii, p. 333.

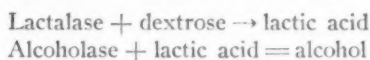
<sup>16</sup> OPPENHEIMER: *Centralblatt für Physiologie*, 1903, xvi, p. 712

<sup>17</sup> SPIRO: *Zeitschrift für physiologische Chemie*, 1877, i, p. 111

<sup>18</sup> ASHER and JACKSON: *Zeitschrift für Biologie*, xli, 3, p. 393.

dogs, could detect no increase in lactic acid due to the sugar. Some attribute the increase in lactic acid to the lack of oxygen caused by the greater proteid destruction.

W. Hutson Ford<sup>19</sup> in 1858 reported experiments in which he demonstrated the presence of alcohol in normal blood. Recently Simáček<sup>20</sup> and Stoklasa<sup>21</sup> have drawn attention to the same phenomena, and others, quoted by Ford in the paper above referred to, confirmed his discovery. Stoklasa explains the formation of lactic acid and alcohol from dextrose as follows:



It is probable that other fatty acids are formed in the glycolysis.<sup>22</sup> The ultimate products would be CO<sub>2</sub> and H<sub>2</sub>O. Locke and Rosenheim<sup>23</sup> found a general parallelism between the amount of sugar lost from the blood during perfusion and the amount of CO<sub>2</sub> formed. The blood coming from the veins of the living perfused leg is very dark in color. In the dead legs, where there was no loss of sugar, the blood coming from the veins remained arterial in color.

The sugars utilized directly by the tissues are those that are directly fermentable by yeast. It is natural to suppose that the products formed would be the same in each case. The enzymes may differ in amount and the results from a quantitative standpoint may vary, but in each case the living cell is the factor to be considered. The oxidation of these sugars seems to be a function of living protoplasm. There is no reason to suspect products qualitatively different from that formed by the action of the yeast cell. This point will be taken up in future work.

#### CONCLUSIONS.

1. The living muscles of an animal when perfused with dextrose, levulose, or galactose cause a rapid oxidation of these sugars.

<sup>19</sup> FORD, W. HUTSON: *Journal of physiology*, 1906, xxxiv, p. 430.

<sup>20</sup> SIMÁČEK: *Centralblatt für Physiologie*, 1904, xvii, pp. 3, 209.

<sup>21</sup> STOKLASA: *Archiv für die gesammte Physiologie*, 1904, ci, p. 311; *Centralblatt für Physiologie*, 1905, xviii, p. 793.

<sup>22</sup> NEF: *LIEBIG'S Annalen*, 1907, ccclvii, p. 214; also, cccxxv, p. 323.

<sup>23</sup> LOCKE and ROSENHEIM: *Loc. cit.*



2. The results with maltose would indicate that little if any of it is oxidized directly by the muscle.
3. Increasing the amount of sugar in the perfused blood increases the amount oxidized.
4. Stimulation of the muscles during the perfusion increases the oxidation.
5. The perfusion of dead muscles shows practically no loss of sugar.
6. Both in living and dead tissues perfusion causes an edematous condition. This occurs very much sooner and to a greater degree in the dead tissues.
7. The perfused liver also utilizes the common sugars. It is probable that this will hold for all glandular organs.
8. The glycogen-storing function of the liver is lost in perfusion much sooner than the sugar-destroying function. The same statement holds for the muscle.
9. The glycolysis occurring in drawn blood at 40° C. in two hours is very small in amount.

## ON GLYCOLYSIS.

By HUGH McGUIGAN.

[From the Laboratory of Physiology and Pharmacology, Washington University.]

THE removal of the pancreas causes death, presumably from the inability of the tissues to use dextrose. The natural inference is that the pancreas elaborates something that is necessary in glycolysis. Brown-Séquard<sup>1</sup> was the first to call such a substance "internal secretion." The mechanism by which this substance aids glycolysis is by no means clearly understood, neither are the chemical nor the physical properties of the substance.

In 1903 Cohnheim<sup>2</sup> published the first of a series of papers giving the results of experiments in which he claimed that while the expressed juice of muscle exerted very little influence on the destruction of sugar, and the extract of the pancreas was equally inert, a mixture of the two possessed powerful glycolytic properties. Since then Cohnheim<sup>2</sup> has published several papers corroborating and extending his first work. The known facts regarding the removal of the pancreas made his theory very plausible, and it was readily accepted. However, there has been much adverse criticism of his work. The work, especially of Claus and Embden,<sup>3</sup> was sufficient to cast considerable doubt on his results. On the other hand, several workers have confirmed him. Probably the most striking confirmation he has received has been by G. W. Hall.<sup>4</sup> This worker not only confirms him, but claims to have isolated the activator from the pancreas extract, which is more

<sup>1</sup> See "Internal secretion and the ductless glands," by SWALE VINCENT, *The Lancet*, August 11 and 18, 1896, for history and literature of internal secretion.

<sup>2</sup> COHNHEIM: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 336; 1904, xlii, p. 401; 1905, xliii, p. 547; 1906, xlvii, p. 253.

<sup>3</sup> CLAUS and EMBDEN: *HOFMEISTER'S Beiträge*, 1905, v, p. 214; 1906, vi, p. 343.

<sup>4</sup> HALL, G. W.: *This journal*, 1907, xviii, p. 280.

active than the extract itself. This activator he found to be specific for dextrose. On the glycolysis of levulose and lactose it exerted no influence. Some work that I have in progress in connection with glycosuria, and glycolysis in living tissues, led me to doubt these results, and, in repeating the work of both investigators, I have been unable to find a trace of the action they describe.

#### METHODS.

The animals were killed by bleeding from the carotid under ether anaesthesia, and the fresh muscles ground in a sausage machine and then in a mortar with sand. After this cold water was added, and in most cases ice was added, and the cold mixture allowed to macerate for about four hours. Any change from this procedure is noted in the protocols. From the mixture the muscle juice was obtained by the aid of a hand press. A definite quantity of this extract was taken and  $\text{NaHCO}_3$ ,  $\text{MgCO}_3$ , or phosphate solution,<sup>5</sup> as indicated, added to preserve neutrality, and sugar added. In every case sufficient toluol was added so that after shaking a distinct layer covered the tops of the liquid. The digestion was performed in small Erlenmeyer flasks at  $40^\circ \text{C}$ . in a thermostat.

At the beginning and end of the experiment the amount of reduction was determined by Fehling's or Pavy's solution, and the amount of glycolysis calculated. Fresh Pavy or Fehling was prepared for each series. The proteid was removed by  $\text{Na}_2\text{SO}_4$  solution and acetic acid; and the precipitate thoroughly washed with  $\text{Na}_2\text{SO}_4$  solution, and all the filtrates of each series were made to the same volume at  $18\text{--}20^\circ \text{C}$ .

#### EXPERIMENTS.

In a series of three experiments on the muscles of two cats and one dog, using the corresponding pancreas and  $\text{NaHCO}_3$  (five P. M.) for neutralization, no evidence of increased glycolysis was shown as measured by Fehling's solution. In the other experiments recorded Pavy's solution was used.

<sup>5</sup> The phosphate solution consisted of nine parts disodium and one part monosodium phosphate 10 per cent strength.

*Experiment 4. — Cat's muscle.*

Three hundred and fifty grams, ground and macerated with 500 c.c. of tap water at room temperature (18° C.) over night, and the juice (630 c.c.), expressed as above, was obtained. The pancreas, weight 6 gm., was boiled and ground in sand, filtered through cheese cloth, and the united filtrates evaporated to dryness on water bath. The residue was extracted with 95 per cent alcohol and filtered through paper. Again evaporated and taken up with water (60 c.c.) 1 gm. equals 10 c.c. The digestion lasted thirty-six hours at 38°–40° C.

Flask number.	Muscle ext. in c.c.	Pancreatic ext. in c.c.
1 . . . . .	75	2
2 . . . . .	75	5
3 . . . . .	75	10
4 . . . . .	75	15
5 . . . . .	75	25
6 . . . . .	75	0

After thirty-six hours the flasks were boiled in water, the proteids removed with  $\text{Na}_2\text{SO}_4$  + acetic acid, and the filtrates made to 250 c.c. 50 c.c. Pavy's solution were used with the following results:

Number of filtrate.	Amount to reduce 50 c.c. c.c.	Amount sugar in filtrate. 250 c.c.	Glycolysis. gm.
1 . . . . .	6.9	0.9058	—,0254
2 . . . . .	6.9	0.9058	—,0254
3 . . . . .	7.0	0.8928	—,0124
4 . . . . .	7.0	0.8928	—,0124
5 . . . . .	7.2	0.8680	.0124
6 . . . . .	7.1	0.8804	....

In this case the sugar in the control flask (6) was not determined at the beginning of, but at the expiration of, the experiment. The amount of glycolysis during the time of the experiment without the addition of pancreatic extract therefore is not known. The experiment is recorded merely to emphasize the limitations of the method under changing conditions. Ordinarily the amount of glycolysis during this time without the addition of pancreatic extract is from 30–60 mgm. dextrose.

If we assume that there was no glycolysis in the control, or that the normal glycolysis was the same in each flask, then in flask Number 5 there was an increase of 12.4 mgm. in the glycolysis. If the other tubes had also shown this amount of increase or more, the contention of Cohnheim might be considered confirmed, but unfortunately the others showed an inhibition of glycolysis of double the amount quoted in the first. Here, again, an inhibition of the glycolysis might be invoked due to too large an amount of the activator, but examination showed that this is not the case. Observation showed that there was only 0.3 c.c. difference in the titrations between the extremes, and any one familiar with sugar titrations knows that this could be accounted for in a number of ways, even with the same solutions, without invoking the aid of an activator.

Pavy's method, when carried out under similar conditions, gives fairly good results, and in work of this kind is sufficiently accurate. The importance of slight differences in results by this method, however, must not be exaggerated, and conclusions of such importance as Cohnheim has drawn should not be based on the results of this method alone, even though the differences were greater than he has reported. Personally, I have found no differences sufficient to necessitate the use of more delicate and more tedious methods.

In working with this method the cleanest results are obtained when the sugar content of the liquid is such that 10 to 20 c.c. of it reduces 25 c.c. of Pavy's solution. In the above experiment the sugar filtrate was too strong to give the best results.

*Experiment 5. — Cat's muscle, cat's pancreas.*

Three hundred and ninety-two grams muscle ground in sausage-grinder and in a mortar with sand were extracted with ice-water for three hours, filtered, and pressed. 530 c.c. of fluid was obtained. Sp. Gr. 1.017. To the whole liquid 5 gm. of dextrose was added, shaken thoroughly, and 50 c.c. taken for sugar determination.  $\text{MgCO}_3$  in substance was added to preserve neutrality. Toluol was added to each flask to form a distinct layer on top. The flasks were closed with cotton and incubated forty-three hours at 40° C. There was no indication of bacterial growth. The alcoholic-watery extract of the pancreas was prepared as above, 2 c.c. equal 1 gm.

Flask number.	Muscle ext. in c.c.	Pancreatic ext. in c.c.
1 . . . . .	50	..
2 . . . . .	50	0.1
3 . . . . .	50	0.2
4 . . . . .	50	0.3
5 . . . . .	50	0.5
6 . . . . .	50	0.7
7 . . . . .	50	1.0
8 . . . . .	50	2.0
9 . . . . .	50	..

The proteids were removed by  $\text{Na}_2\text{SO}_4$  and acetic acid, and each of the filtrates made to 500 c.c.

In the filtrations 25 c.c. Pavy's solution was used in each case.

Number.	Filtration amount. c.c.	Sugar. per cent.	Glycolysis. gm.
Beginning of experiment .	14.0	0.8930	....
1. After 43 hours . . . .	15.5	0.8065	0.0865
2. After 43 hours . . . .	15.0	0.8333	0.0597
3. After 43 hours . . . .	15.3	0.8170	0.0760
4. After 43 hours . . . .	16.0	0.7812	0.1118
5. After 43 hours . . . .	14.7	0.8503	0.0427
6. After 43 hours . . . .	16.0	0.7812	0.1118
7. After 43 hours . . . .	15.9	0.7862	0.1078
8. After 43 hours . . . .	17.5	0.7143	0.1767
9. After 43 hours . . . .	15.5	0.8930	0.0865

The results here could be construed to confirm the glycolytic theory, and would call for the application of different methods were other factors not to be considered. Wider and apparently more inconsistent differences than these are reported by Cohnheim,<sup>6</sup> for which he has no satisfactory explanation to offer. I believe the results given above are due to the varying growths of bacteria in the solutions. An example will illustrate the probability of this assumption.

*Experiment 6.* — Ox muscle with ox pancreas.

Flesh was obtained from the slaughter-house which had been killed some hours before. Six hundred grams was extracted with 800 gm. ice-water, after grinding as above. The extraction lasted

<sup>6</sup> COHNHEIM: Zeitschrift für physiologische Chemie, 1906, xlvii, p. 280.

twelve hours at an outside temperature of  $2^{\circ}$  C., and contained ice during the extraction; 970 c.c. fluid sp. gr. 1.016 was obtained. After the addition of dextrose 50 c.c. of this solution was used in each test. The pancreas solution was such that 10 c.c. equalled 10 gm. pancreas.  $\text{MgCO}_3$  was used to neutralize, and toluol was added as before. The sugar content at the beginning of the experiment was 0.9800 per cent.

Flask number.	Pancreas.	Sugar. per cent.	Glycolysis. gm.
1 . . . . .	..	0.9060	0.0740
2 . . . . .	0.1	0.9060	0.0740
3 . . . . .	0.2	0.9060	0.0740
4 . . . . .	0.5	0.9060	0.0740
5 . . . . .	0.8	0.9190	0.0610
6 . . . . .	1.0	0.9060	0.0740
7 . . . . .	2.0	0.9060	0.0740
8 . . . . .	3.0	0.9060	0.0740
9 . . . . .	..	no toluol added	0.9800

The digestion was continued forty-two hours at  $40^{\circ}$  C. The objection may be made that too much pancreatic extract was added. The particular feature of the experiment is the entire loss of the sugar in Number 9, the sample where no toluol was added. This sample smelled foul. None of the others showed any signs of putrefaction. The loss of several milligrams sugar, however, is highly probable without any sign of foulness, and where such loss occurs in work of this kind it is absolutely necessary to eliminate the probability of a bacterial growth before assigning any special importance to the co-operative action of the tissue juices. Other experiments where smaller quantities of pancreatic extract was used showed no increase in glycolysis.

*Experiment 8.* — Cat's muscle with cat's pancreas.

Three hundred and ten grams fresh muscle ground in sand as above and macerated four hours with 400 c.c. distilled water at  $0^{\circ}$  to  $2^{\circ}$  C.; 425 c.c. filtrate was obtained after pressure. The muscle residue was ground again with cold water and filtered, and the united filtrates (425 c.c.) used in the experiments.

The phosphotungstic acid precipitate of the pancreas extract was prepared exactly as Hall recommends. The very slight excess of acidity after the precipitation of the excess of  $\text{Ba}(\text{OH})_2$  was neu-



tralized with  $\text{NaHCO}_3$ . The amount of  $\text{NaHCO}_3$  used was 0.1 gm. to a volume representing 40 gm. of pancreas. Ten c.c. of the final pancreatic extract represented 2 gm. of fresh pancreas.

Two series of experiments were carried out. In one the phosphate solution (10 per cent solution 9 parts disodium, 1 part monosodium) recommended by Hall was used to neutralize one series, and  $\text{MgCO}_3$  in substance to neutralize the other. The following experiments were carried out with these preparations:

Flask number.	Muscle ext. c.c.	Pancreas ext.	Phosphate sol. c.c.	Sugar found. per cent.	Glycolysis. mgm.
1 . . . . .	50	1	20	1.021	29
2 . . . . .	50	2	20	1.020	30
3 . . . . .	50	3	20	1.016	34
4 . . . . .	50	4	20	1.020	30
5 . . . . .	50	6	20	1.021	29
6 . . . . .	50	8	20	1.021	29
7 . . . . .	50	10	20	1.024	26
8 . . . . .	50	..	20	1.024	26

Sugar at commencement of experiment, 1.050.

Toluol was used as before. The digestion continued forty-three hours at 40° C.

Flask.	Muscle ext. in c.c.	Pancreas ext. in c.c.	Sugar. per cent.	Glycolysis. mgm.
A . . . . .	50	..	1.016	34
B . . . . .	50	1	1.020	30
C . . . . .	50	2	1.024	26
D . . . . .	50	3	1.024	26
E . . . . .	50	8	1.024	26
F . . . . .	50	15	1.028	22

*Experiment 9.* — For this series the muscles of a young dog were taken.

Seven hundred grams after grinding were macerated with 800 c.c. ice-cold distilled water for four hours; 950 c.c. of filtrate was obtained, and this was made to 1000 c.c. and about 10 gm. dextrose added. Sugar determined at once amounted to 0.9843. The pancreas weighed 17 gm. This was prepared according to the method recommended by Hall, and was made so that 10 c.c. equalled 1 gm. fresh pancreas.

Flask number.	Muscle ext. in c.c.	Pancreas ext. in c.c.	To neutralize.	Glycolysis in gm.
1 . . . . .	50	..	MgCO <sub>3</sub>	0.1102
2 . . . . .	50	0.1	MgCO <sub>3</sub>	0.1102
3 . . . . .	50	0.2	MgCO <sub>3</sub>	0.0915
4 . . . . .	50	0.4	MgCO <sub>3</sub>	0.1510
5 . . . . .	50	0.6	MgCO <sub>3</sub>	lost
6 . . . . .	50	1.0	MgCO <sub>3</sub>	0.0583
7 . . . . .	50	2.0	MgCO <sub>3</sub>	0.0583
8 . . . . .	50	4.0	MgCO <sub>3</sub>	0.1041
9 . . . . .	50	6.0	MgCO <sub>3</sub>	0.0619
10 . . . . .	50	10.0	MgCO <sub>3</sub>	0.1041

With the same solutions the following tests were made, using the phosphate solution to neutralize, 15 c.c. in each flask:

Flask.	Muscle ext. in c.c.	Pancreas ext. in c.c.	Glycolysis in 21 hours in gm.
A . . . . .	50	..	....
B . . . . .	50	0.1	—0.0038
C . . . . .	50	0.2	—0.0019
D . . . . .	50	0.3	—0.0019
E . . . . .	50	0.4	—0.0038
F . . . . .	50	0.5	—0.0077
G . . . . .	50	0.6	—0.0038
H . . . . .	50	1.0	—0.0038
I . . . . .	50	2.0	—0.0019

In the series to which MgCO<sub>3</sub> was added there was a probable bacterial growth to account for the varying degrees of glycolysis. There is an apparent inhibition of glycolysis where the phosphate solution was used, but I consider this well within the limits of error of the method.

#### CONCLUSIONS.

The experiments recorded show that mixtures of muscle and pancreatic extracts are as inert in glycolysis as is the muscle extract alone.

## STUDIES IN RESUSCITATION.—II. THE REFLEX EXCITABILITY OF THE BRAIN AND SPINAL CORD AFTER CEREBRAL ANÆMIA.<sup>1</sup>

By F. H. PIKE, C. C. GUTHRIE, AND G. N. STEWART.

[From the Hull Physiological Laboratory, the University of Chicago.]

WE have given, in other papers,<sup>2</sup> the main phenomena in the resuscitation of the central nervous system after cerebral anæmia. In the present paper we wish to present certain new data on the reflex excitability of the brain and spinal cord during and after the ligation of the head arteries of an animal. The method of producing cerebral anæmia has already been described, and need not be repeated here.

The anterior part of the spinal cord becomes totally inexcitable during the period of anæmia, but may regain its excitability more or less completely when the circulation through it is re-established, and may for a time even become hyperexcitable; *e. g.*, striking the fore paws may cause a much greater reflex response than would normally occur, and a comparatively slight stimulus may cause prolonged scratching, such as might occur in a spinal animal. This hyperexcitability affects also the posterior part of the cord. Cold — *e. g.*, placing the animal on ice — generally has but little effect on the reflex excitability of the cord, but ether subdues it. Again, the anterior part of the cord may never completely regain its normal excitability, but may recover in part. The fore limbs, for example, may contract on the side which is stimulated, but the animal may never recover so that the reflex crosses to involve the opposite fore limb. The hyperexcitability disappears in the animals which

<sup>1</sup> The first of these studies, covering the general conditions affecting resuscitation and the resuscitation of the blood and of the heart, will appear in "The journal of experimental medicine," 1908, x, No. 3.

<sup>2</sup> STEWART, GUTHRIE, BURNS, and PIKE: *Journal of experimental medicine*, 1906, viii, p. 289; GUTHRIE, PIKE, and STEWART: *This journal*, 1906, xvii, p. 344; STEWART and PIKE, *Ibid.*, 1907, xix, p. 328; xx, p. 61; STEWART, *Ibid.*, xx, p. 407.

recover completely. Occasionally, also, the posterior part of the cord never becomes irritable in more than slight degree. The asphyxiation of the posterior part of the cord produced by stoppage of the heart, as we mentioned in the first of these studies, may cause no movements whatever.

The eye reflexes may all return in favorable cases. Etherization of an animal several hours after the release of the cerebral arteries may cause nystagmus just as in a normal animal. Nystagmus may occur also in the resuscitation period when no ether is given.

Six and one-half hours after release from an occlusion of thirty-one minutes, the eye movements (in nystagmus) were observed in the intervals between spasms. Twenty minutes later these movements were continuous, with a rate of fourteen in the minute. The eyelids were opened and the eyeballs were rotated. The nictitating membrane was protruded, but did not take part in the movements. The corneal reflex may sometimes return before the ear reflex appears, or may return in one eye before it appears in the other. In one experiment the eyelid reflex returned so that touching the left eyelid caused a movement of both lids, but touching the lid of the right eye, whose pupil was much more dilated than the left, caused no such movements. The right carotid artery had been used for making a blood-pressure tracing. The corneal reflex subsequently returned in the right eye. In another case the corneal reflex and lachrymal secretion returned in the right eye sooner than in the left, although the right carotid was used for blood pressure. The corneal reflex returned in the left eye about five minutes later.

The ear reflexes sometimes returned before all the eye reflexes appeared, but not invariably. Touching the interior of the ear, flicking it with the finger, or blowing into it caused movements of the eyelid on the same side one hour after starting the heart by massage following a stoppage of fifteen to twenty minutes, with a previous occlusion of twelve and three-fourths minutes. The lid reflex of the eye was good at this time, and the corneal fair. The corneal reflex reappeared, in one instance, four and one-half minutes after release from an imperfect occlusion of thirty minutes, and before any ear reflex was present. A good ear reflex was present two minutes and fifty seconds after release from an occlusion of nine minutes and fifty seconds. There were no eye reflexes present. The natural respiration was exactly synchronous with the artificial. Fifty-nine minutes after release from an oc-

clusion of twenty minutes, the animal (cat) winked its eye on the same side when the ear was flicked, but there was no ear reflex. Twenty-three minutes after release from an occlusion of ten minutes there was a reflex contraction of the fore leg on the same side struck. Eight minutes later both fore legs contracted on striking one of them. In another ten minutes flicking one ear caused movements of that ear and drawing up of both fore legs.

Stimulation of the corner of the mouth three hours and fifty minutes after release from an occlusion of fourteen minutes caused opening and closing movements of the mouth, and movements of the whiskers. Stimulation of the eyelids at this time caused closure of the lids.

Two hours after release from an occlusion of sixty-five minutes the penis was continually executing rapid rhythmical movements (see protocol of Experiment 5).

The rectal sphincter also shows increased irritability during the resuscitation period. Fourteen minutes after release from an occlusion of sixteen minutes the insertion of a thermometer into the rectum caused good kicking movements of the hind limbs. There were also rhythmical movements of the rectal sphincter at the rate of about twelve in the minute. The rectal temperature was  $35.1^{\circ}\text{C}$ . Movements of the hind limbs on inserting a thermometer into the rectum during the resuscitation period are of common occurrence, and both hind limbs are usually involved to an equal extent.

In one experiment, two and one-half hours after release from an occlusion of forty-five minutes, the insertion of a thermometer caused movements of the left hind leg only. Striking the right hind leg caused movements of that leg, but better movements of the opposite leg. The observation was repeated several times. The left leg gave better movements on pinching than the right one gave when it was pinched. The rectal temperature was  $38.2^{\circ}\text{C}$ . The reflex contractions of the rectal sphincter were present, in another experiment, four minutes after release from an occlusion of sixty minutes.

The fore limbs entirely lose their tonicity and reflex excitability during anæmia of the brain, and may gradually regain it during the resuscitation period. Relatively early after the re-establishment of the cerebral circulation, whether by releasing the arteries or starting the heart after temporary stoppage, trembling movements and extensor spasms may appear. The clonic movements seen

during the spasms are of later development. The extensor spasms and trembling movements are of central origin, since they cease on section of the brachial nerves. Scheven<sup>3</sup> and others are inclined to regard some of these phenomena as instances of decerebrate rigidity.

*Experiment 1.*<sup>4</sup> March 23, 1905. — The heart was started by direct massage after a stoppage of about twenty-two minutes. One hour afterward there were spasmodic trembling movements of both fore limbs, superposed upon the slight tonic spasm present at this time. Striking the fore limbs increased the trembling. An hour later all the nerves from the right brachial plexus that could be found going to the right fore limb were divided. The trembling and all other movements ceased permanently. Stimulation of the peripheral end of the brachial nerves caused strong contraction of the fore limb. Stimulation of the central end was without effect. No respiratory movements had yet been seen.

The trembling may spread to involve the hind limbs also. The extensor spasms may appear before any reflex response can be elicited on striking the fore limbs, but striking the fore limbs at such a time may increase the severity of the spasms.

Resuscitation may never proceed far enough to restore the commissural path, and the fore-limb reflex may never cross. After an occlusion of forty-five minutes, for example, the crossing of the fore-limb reflex never occurred, even twelve hours after release, although reflexes were easily obtained on the same side. The time relations of the crossing of the fore-limb reflexes have been given in our first paper on the subject,<sup>5</sup> and need not be considered further here.

The rate of the clonic movements of the fore limbs will be given in the section on resuscitation of the muscles to appear later. Extensor spasms at the rate of ten in the minute, alternating with eleven flexions, were seen fifty-six minutes after release from an occlusion of thirty-one minutes. This rate continued unchanged for fifty minutes.

Closely associated with the fore-limb reflexes are the intercostal reflexes, some of which we have described in a previous paper.<sup>6</sup> This is shown in

<sup>3</sup> SCHEVEN: *Archiv für Psychiatrie*, 1904, xxxviii, p. 926.

<sup>4</sup> The experiments are numbered consecutively for convenience in reference. The chronological order is indicated by the date given.

<sup>5</sup> *Journal of experimental medicine*, *loc. cit.* p. 310.

<sup>6</sup> STEWART and PIKE: This journal, *loc. cit.* p. 339.

*Experiment 2. May 16, 1905.* — An occlusion of twenty-one minutes was followed by stoppage of the heart for about ten minutes. Three hours later tapping the ribs on the right side caused extensive contraction of the intercostal muscles and fore limb on that side, but these were sharply limited in the tailward direction at the level of the diaphragm. At this time the fore-limb reflexes elicited by striking did not cross, but distinct crossing occurred one hour later. A slight spasm of the fore limbs was caused, in another experiment, during the resuscitation period by scratching the inner wall of the thorax over or between the ribs, one hour and forty minutes after release from an occlusion of eighty-one minutes.

The hind limbs and tail execute strong movements during the early part of occlusion, but these movements often, in fact usually, cease before the end of the occlusion if this is at all prolonged, and the hind limbs no longer contract on striking them. Reflex movements of the rectal and vaginal sphincter often persist longer than the hind-limb reflexes, sometimes throughout the period of occlusion.

As an experiment in point we cite Number 4. The reflex movements of the hind legs do not always cease, even during a long occlusion, *e. g.*, eighty-one minutes, but may weaken for a time and then increase in intensity toward the end of the occlusion. These phenomena are shown in Experiment 3.

*Experiment 3. April 15, 1905.* — Large male cat. Ether. Cannula in trachea.

3.07 P. M. Opened chest.

3.20 P. M. Clamped innominate and left subclavian arteries proximal to all branches.

3.26.50. Respiratory movements continue. No reflexes. Movements of hind limbs, which are flexed and extended alternately many times in succession.

3.33 P. M. No more gasps or breathing movements of the abdominal muscles. No movements of fore limbs were observed at all, but there were rapid and strong movements of hind limbs, as before.

3.37 P. M. Hind-leg movements are less strong and rapid. When the leg is pulled out, it resists and is drawn up strongly.

3.39 P. M. Pulse 150. The rectal sphincter contracts rhythmically and strongly on thermometer, the hind legs kicking out when it is introduced.



3.44 P. M. Right hind leg is moving as before, but slower and weaker. Pulled up quite strongly on being stretched.

3.54 P. M. Draws up leg strongly on pulling it.

4.00 P. M. Put thermometer in rectum. Hind legs kick vigorously while thermometer is being put in. Rectal temperature  $35.8^{\circ}$  C.

4.06 P. M. On pulling out the left hind leg get excellent clonic movements. The right hind leg is not now drawn up, as before, on being pulled out.

4.14 P. M. Strong movements of the hind legs on pulling them, as before, and movements of the whole rump.

4.19 P. M. Kicked vigorously on putting thermometer in rectum.

4.33 P. M. Stimulated the right sciatic nerve, which had been exposed without dividing. Slight fall of blood pressure. Strong contraction of right hind leg and weaker contraction of left hind leg. No contraction of the fore legs.

4.38 P. M. Excellent clonus of left hind leg on keeping it extended.

4.41 P. M. Released the head vessels.

Two hours and twelve minutes after release of the head arteries in the above experiment, the aorta was compressed above the diaphragm by pressure of the fingers, and held shut for two minutes. On releasing it the cat at once kicked up its hind end, as far forward as the diaphragm.

Contraction of the hind limbs on touching the anal orifice with a thermometer is of common occurrence in the resuscitation period. It was noted in one experiment about six hours after release from an occlusion of thirty minutes, and in another case four hours after release from an occlusion of sixty minutes. Other instances have been cited in the paragraph on the reflexes of the rectal sphincter.

Struggling movements of the hind limbs were observed in one experiment six minutes after occluding the head arteries, — a somewhat unusual phenomenon, — but no reflexes could be elicited from the hind limbs after release of the vessels thirty-five minutes later. The anal reflex was also absent.

Movements of the tail are usually associated with movements of the hind limbs. The tail generally moves slowly and quietly to and fro, but the movements increase in frequency and violence during the spasms. The tail hairs and those of the back, as has been mentioned, are often erected continuously during the time of the spasms.

A peculiar type of hind-limb reflex observed during the resuscitation period is the scratch reflex. It occurs well on in the resuscitation period following prolonged occlusion, and is in all respects similar to the scratch reflex appearing in the spinal animal after recovery from shock. We have previously reported an experiment<sup>7</sup> in which the scratch reflex was elicited after section of the brain stem by pinching the ear. Pinching the ear also caused the scratching movements in another case in which the brain stem was anatomically intact, but had suffered anæmia of fourteen minutes' duration. We give the protocol.

*Experiment 4. March 27, 1905.* — Half-grown male cat. Ether. Tube in larynx.

- 4.24 P. M. Occluded head arteries.
- 4.28.30. Hind legs stretching out.
- 4.29.45. Strong gasping respiration. Hind legs move. No eye reflexes. Eyes widely dilated.
- 4.31 P. M. No more respiratory or leg movements, or movements of any kind after this.
- 4.38 P. M. Released vessels.
- 4.38.15. Movements of trunk and limbs and then no more.
- 4.58 P. M. One deep respiration.
- 5.01 P. M. Pupils considerably narrower. Strong respiratory movements, one in eight seconds, then quickening to one in six seconds.
- 5.14 P. M. Strong extensor spasm of fore limbs, which remain extended.
- 5.15 P. M. Strong general spasms with marked opisthotonus, the head being strongly extended. Good corneal reflex. In no animal yet observed have spasms been so severe at such a short time after release of vessels. No laryngeal difficulty. As it seemed highly unlikely that the animal would live long if the spasms were not combated, a little chloroform was given. The spasms relaxed, but respiration soon stopped and the pupils dilated. Amount of chloroform given could not have affected a normal animal. Perhaps in the injured condition of the brain and the upper part of the cord even a small dose produces a profound effect. Set up artificial respiration.
- 5.32 P. M. The animal is breathing. No corneal reflex. Stopped artificial respiration.
- 5.44 P. M. Pupils absolutely closed.

<sup>7</sup> STEWART and PIKE: This journal, 1907, xx, p. 70.

5.58 P. M. Animal stopped breathing. Began artificial respiration.

6.15 P. M. Fore limbs extended. They had been lax. No corneal, lid, light, or ear reflex. On striking hind or fore limbs, get movements of corresponding limb on opposite side. Pulse 160 a minute.

6.27 P. M. Temperature in rectum  $33.5^{\circ}$  C. When thermometer was introduced, the hind legs kicked and efforts at breathing were made.

7.15 P. M. Vigorous scratching movements of left hind legs suddenly began. Between 7.15 and 7.25 there were three more periods of scratching.

7.25 P. M. No eye reflexes. Twisting of neck occurs with the leg movements. The extensor spasm had disappeared from the fore limbs. From 7.25 to 7.45 there was practically continuous scratching.

7.55 P. M. Strong lashing movements of tail and movements of hind legs terminated by general contraction of abdominal muscles which expelled some feces.

7.56 P. M. Strong rapid scratching movements of right legs. Pupils dilated very much to about half maximal. Immediately before they were strongly contracted.

8.05 P. M. Strong rapid scratching movements of left leg. Goes on almost continuously for eight minutes.

8.16 P. M. Striking the hind legs causes flexor movements of them. Striking the rump or hips causes extensor movements of the hind legs. Striking the fore legs produces small effect. The fore legs are soft, having no rigor or extensor spasms.

8.34 P. M. Strong scratching movements of right hind legs. Turning of head to left. This, which is the first head movement seen for some time, was repeated two or three times.

8.37 P. M. Very strong scratching movements of left hind leg, then of both hind legs, then a spasm of whole posterior end of body. The scratching movements are very extensive, the feet extending up to the chest, as if directed to remove some feeling of discomfort or obstacle to respiration. This was also seen in all the scratching movements previously noticed.

8.42 P. M. Stopped artificial respiration for a few seconds, to see if breathing would take place. It did not, so far as chest and upper end of the body were concerned, but the movements of the abdominal wall and hind legs were greatly increased. The fore limbs did not participate in the movements.

9.00 P. M. Pinching the ear seems to cause movements of hind legs; repeated three times. Later on this could not be got.

9.20 P. M. Stimulated central end of left sciatic. There were strong contractions of right hind leg and fair of left fore leg. Could not see any contraction of the right fore leg. No head contractions.

9.23 P. M. Divided spinal cord in neck. Strong contraction of whole of the body below the section when the cut was made.

9.55 P. M. Stimulated upper end of right vago-sympathetic with usual effect on the eye. Slight scratch movements of left hind leg.

10.10 P. M. Stimulated upper end of left sciatic. Causes drawing up in flexion of both hind legs. They are kept in this position during the stimulation. When the stimulation is stopped, they kick down several times in succession, being alternately quickly extended and then flexed.

10.30 P. M. Stopped artificial respiration. In one and one-half minutes, movements of the abdominal muscles like respiratory movements appear; also movements of the skin of the trunk and rump. Then, in one-half minute more, a very strong extensor spasm, especially of hind legs, but involving also the fore limbs. Then the limbs come back to their original position. Experiment discontinued.

It is noteworthy, in this connection, that although the scratch reflex could be obtained for a time by pinching the ear, it could not be got later on. It should be remarked, too, that section of the cord had but little effect upon the reflexes in the part below the level of the section.

The rate of the scratching movements, about five per second, appears from the second protocol which we submit.

*Experiment 5. May 23, 1905.*—Adult male cat. Ether. Tube in larynx.

2.19 P. M. Occluded head vessels in usual manner.

2.32 P. M. Put temporary ligature around the innominate distal to origin of the left carotid.

2.41.50. Strong respiratory gasps.

2.42.10. Another strong respiratory gasp. Strong struggles. Kicking with hind legs. Urinated. No eye reflexes. Pupils wide.

2.44 P. M. Struggles less, but still kicking gently with right hind leg. No gasps.

2.48 P. M. Pulse 171.

2.56 P. M. Pulse 180.

3.22 P. M. Pulse 138.

- 3.24 P. M. Released head vessels.
- 3.54 P. M. First gasp; strong.
- 4.03 P. M. Trembling movements of the fore limbs.
- 4.04 P. M. Stopped artificial respiration. Breathing goes on 24 in the minute. Pupils are not diminished much.
- 4.25 P. M. No eye reflexes. Twitching of eyelids and also general twitching spasms of the neck, shoulders, and thorax. Natural respiration 44 per minute.
- 4.51 P. M. Scratching movements of left hind leg.
- 4.55.30. Left pupil now narrow, but wider than normal; right pupil quite wide. Strong convulsive movements of lower end of body, and kicking of hind legs. When these relaxed, it was seen that the natural breathing had stopped completely. Heart still beating. Started artificial respiration immediately. Both pupils now dilated.
- 4.59 P. M. Slight gasping movements of mouth.
- 5.02 P. M. Stopped artificial respiration. A little breathing, but not enough to be effective. Started artificial respiration again. Pulse 184. Twitching over right shoulder. Tears in both eyes, but especially in left. Good corneal tension.
- 5.21.30. Good gasps. Stopped artificial respiration. Cat goes on breathing for a minute or so, but had to start artificial respiration again. Almost immediately after starting artificial respiration there was strong kicking of the hind legs. Good secretion of saliva going on, and has been going on for a long time, as shown by wiping the mouth dry. It trickles copiously from the mouth. Rapid rhythmical movements of the penis are going on continuously. Rectal temperature  $36.3^{\circ}$  C.
- 5.54.30. A slight gasp. Followed by flexion and abduction of left hind foot rhythmically for two or three seconds.
- 5.56 P. M. Several more gasps and moderately strong spasms of hind limbs, the left one scratching vigorously.
- 5.58 P. M. Another gasp, and strong scratching of left hind leg. Another in about two seconds, with flexion of trunk and stiffening of fore limbs in extension.
- 6.00 P. M. Scratching spasm of left hind leg.
- 6.00.40. Scratching spasm of left hind leg. Each spasm of scratching lasts about two to three seconds.
- 6.05 P. M. Four more scratching spasms of left hind leg. About fifteen movements during each spasm.
- 6.07.40. Eight more scratching spasms, becoming almost continuous.
- 6.15 P. M. Pulse 204 a minute. Slow rotatory movements of left hind foot almost continuously.

7.05 P. M. Pulse 160. Reflex excitability of lower part of cord is high, but cervical cord and brain do not now seem to be discharging at all. Striking the fore limbs causes no response. Rectal temperature  $37^{\circ}$  C. Artificial respiration is still maintained. No attempts at breathing. Good secretion of saliva still going on. Scratching movements of left hind foot almost constant.

7.48 P. M. Pulse 171 and strong. There have been many strong fits of scratching movements of the hind legs, with the fore limbs stiffened in rather weak extensor spasms during the strongest of them. Salivary secretion still going on. Tongue and eyelids excitable to direct stimulation. Pupils wide, and cornea slack.

7.53 P. M. Marked extensor spasms of fore limbs, as well as contraction of hind limbs, came on suddenly and lasted only a few seconds. Cornea tense, and pupils at maximal dilation.

8.03 P. M. Stopped artificial respiration for about one minute. The animal did not breathe, and the fore limbs were stretched in extensor spasm. Started artificial respiration again. Fore limbs now execute trembling movements.

8.15 P. M. Stopped artificial respiration. In seventeen seconds the fore limbs were stretched in extensor spasms. The heart was markedly slowed, and in one minute it could no longer be felt. No contraction of hind limbs or abdomen. Started artificial respiration again. Hairs on tail erected.

8.18 P. M. Repeated with same results.

8.21 P. M. Stopped artificial respiration. No effect observed even in four minutes. Heart had evidently stopped in last observation.

In neither of these experiments did the function of the higher nervous centres return in more than a slight degree. The respiration did not return permanently, nor continue uninterruptedly in either case. The cord must therefore have been very free from the impulses coming down from the brain, and must have approached closely the condition which follows anatomical section.

An instance of apparently complete regeneration of the sciatic nerve after section was found in one experiment. By mistake a cat whose left sciatic nerve had been cut in November, 1904, was used for operation on June 6, 1905. Two hours after release from a total occlusion of forty minutes, immediately preceded by a partial occlusion of seven and three-fourths minutes, scratching movements of both right and left hind legs occurred.

The spasms described in our first paper involve both fore and hind limbs. These spasms are of central origin, although they

appear when the brain stem has been divided at the level of the roots of origin of the fifth nerve. Transection of the cord in the mid-dorsal region stops all the spasms below the level of the section.

In one experiment the spinal cord was divided just below the junction of the last rib with the vertebral column, three hours after release from an occlusion of forty minutes. There were strong movements of both fore and hind limbs at the moment of division, and contraction of the neck, thorax, and abdomen. No spontaneous movements of the hind limbs occurred after this, and although they contracted on pinching six minutes after division of the cord, these contractions were not nearly so strong as the contractions similarly elicited before division. The fore limbs, on the contrary, seemed to contract reflexly better than before. Twenty-two minutes after division of the cord artificial respiration was stopped. In forty-five seconds shallow gasps appeared, then head and neck movements, and strong extension of the fore limbs, with each attempt at respiration. No movements of the hind limbs or of any part of the body below the level of the section of the cord occurred until one minute and forty-five seconds had elapsed since the stoppage of artificial respiration, when the tail moved. In two minutes and ten seconds after the beginning of asphyxia there was strong extension of the hind limbs, at a time when all contraction had passed away from the part of the body anterior to the section.

The absence of the profound shock usually attending such a procedure in the part of the cord posterior to the section is noticeable also in this experiment. Convulsions of the posterior part of the animal during asphyxia at a time when the anterior part of the animal is motionless are of not infrequent occurrence. The damaged portion of the cord succumbs to asphyxia and, as we have pointed out elsewhere, to strychnine<sup>8</sup> sooner than the posterior uninjured part.

The effect of the synapses in delaying the return of a reflex is well shown in the case of the cardio-inhibitory mechanism, and the limitation of a fore-limb reflex to one side is doubtless due to the failure of the re-establishment of conduction at the synapses in the commissural pathway. It is difficult to interpret these phenomena on any hypothesis which does not postulate a fairly well-marked physiological individuality of the nerve cells, and a more or less definite break between two cells at the synapsis. It is diffi-

<sup>8</sup> *Journal of experimental medicine, loc. cit.* p. 309.



cult to see how these conditions could be met if there were a fluid connection between the two cells joined by the synapse.

It will be noted, from a consideration of the preceding paragraphs, that we have been able to produce, by the method of cerebral anæmia and without effecting the anatomical rupture of any aborally conducting pathways, many, if not all, of the phenomena of spinal shock. For example, the reflexes of the skeletal muscles of the posterior part of the animal commonly disappear before the end of the occlusion period, while the anal and vaginal reflexes in the female are relatively little affected. Occasionally, however, the latter also disappear. Vaso-motor reflexes are difficult to elicit by stimulation of the central end of the sciatic nerve. When the period of anæmia has been sufficiently prolonged to cause severe injury to the encephalon and anterior part of the cord, the animal falls into much the same condition as a "spinal" or "bulbo-spinal" animal. The scratch reflex was elicited as strongly and as certainly in some of our animals as in the spinal animal. And furthermore, when the reflexes, temporarily depressed during the anæmia, returned in such animals, transection of the cord below the lower level of the anæmic region has no more effect on them than a second transection of the cord in a spinal animal which has recovered from the depression attending the first transection.

These results were wholly unlooked for, and their bearing upon the mechanism of spinal shock was not at first recognized. The statement of previous authors, *c. g.*, Asher and Lüscher,<sup>9</sup> is that shock is not produced by anæmia of the brain. The results just quoted show such a close and striking correspondence to those following spinal transection that we are inclined to regard the two conditions as, in all essential points, identical. Barring a possible but extremely doubtful stimulation of descending inhibitory pathways by the anæmia, it would appear that the only necessary condition for the production of spinal shock is the cutting off of the higher reflex pathways through the upper part of the medulla and brain. More upon this subject cannot be stated here, but other experiments, devoted to working out the details of spinal shock, are now in progress, and we hope soon to offer more upon its mechanism and significance as well as upon its bearing upon the problem of the functions of the spinal cord.

<sup>9</sup> ASHER and LÜSCHER: *Zeitschrift für Biologie*, 1899, xxxviii, p. 499.

**CORRECTION.**

In the Proceedings of the Physiological Society, page xxix, line 15, (*a*) *Proteins* should read (*a*) *Proteans*.





## DIRECT AND REFLEX RESPONSE OF THE CARDIO-INHIBITORY CENTRE TO INCREASED BLOOD PRESSURE.<sup>1</sup>

By J. A. E. EYSTER AND D. R. HOOKER.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

### HISTORICAL REVIEW.

MAREY in 1859 (1) first observed the decrease in pulse rate that accompanies a considerable rise of the arterial blood pressure in warm-blooded animals. He explained this phenomenon as due to a peculiarity of the heart muscle whereby it is able to develop always the same energy in a given unit of time. With the rise of blood pressure more work in each individual beat was thrown upon the heart, and to compensate for this the heart rate became slower. Bernstein in 1867 (2) showed, however, that this slowing was not present after section of both vagus nerves, at least not to the same degree as when these nerves were intact. Following the work of Bernstein, a large number of investigations have been carried out for the purpose of determining the following points. In the first place, what effect, if any, increase of arterial blood pressure produces upon the heart rate when a part or all of the extrinsic cardiac nerves are cut, and whether the slowing that occurs in the normal heart with full extrinsic nerve supply is due to a direct effect of the rise of blood pressure upon the cardio-inhibitory centre, or whether the centre is affected through afferent nerves arising within the heart or other part of the vascular system, and the slowing is thus brought about reflexly. The literature that has accumulated is very large and in great part contradictory. In regard to the first point, all authors agree that slowing of the heart as a result of a rise of arterial pressure, if it occurs at all,

<sup>1</sup> A preliminary report of this work was made before the American Physiological Society in May, 1907 (Proceedings of the American Physiological Society, This journal, 1907, xix, p. xii). An abstract of the first part of the work was published in the *Zentralblatt für Physiologie*, 1908, xxi, p. 1.

is much less in degree after section of the vagi than with these nerves intact, and since the work of Bernstein the rôle of the vagi in the phenomenon has been accepted. An excellent résumé of the older literature is given by Tigerstedt (3), and of the more recent works by Hofmann (4). Only certain articles of fundamental importance and very recent ones will be referred to in this paper.

Nawrocki in 1870 (5) confirmed the importance of the vagi and expressed the phenomenon in terms of increase and decrease of the tonus of these nerves as a result of the changes in blood pressure. Several years later the same author (6) investigated the effect of changes in blood pressure produced by occlusion of the aorta, by section of the splanchnics, stimulation of depressors, bleeding, injection of defibrinated blood, and by stimulation of the cut spinal cord and various sensory nerves, in animals with (a) sectioned vagi but intact cord and sympathetics, (b) sectioned vagi and cord, and (c) sectioned vagi, cord, and sympathetics. These experiments were performed upon dogs, cats, and rabbits. He found no noteworthy or constant changes of pulse rate under any of these conditions. V. Bezold and Stezinsky (7) several years previous to this had found an increase of pulse rate with rise of pressure up to a certain point after section of the vagi, and Knoll (8) had found no change under these conditions. François-Franck (9), on the contrary, found a slight decrease. Observations since this time have accumulated upon both sides of this question, some authors finding no change of pulse rate as a result of rise of pressure after section of the vagi or all the extrinsic cardiac nerves, others an increase or slight decrease of rate (for full literature see Herlitzka (10)). Johansson (11) found an increase in rate with rise of blood pressure in the mammalian heart with vagi and accelerators cut, but only if the rise of blood pressure were rapid, — equal to 1 mm. or more of mercury per second. Following the rise of pressure, the increased rate lasted only a short time; the previous rate then returned, or the heart was even slower than normal.

With all the extrinsic nerves intact except the vagi, a number of observers have observed slight slowing of the heart accompanying sudden and great rises of arterial pressure. This slowing, when present, however, was inconstant, and relatively much less than before section of those nerves.

In the cold-blooded heart, artificially fed but with intact vagi, the observations of Tschirjew (12), Ludwig and Luchsinger (13), and

Sewell and Donaldson (14) tend to show that variations in venous pressure cause a change in rate, — an increase of rate with rise of venous pressure. Marey (15) observed an increase of rate in the isolated turtle's heart with rise of arterial pressure, the venous pressure being kept constant. Sewell and Donaldson (14) found, however, that changes in intraventricular pressure produce no influence, pressure variations within the sinus alone being effective, and Howell and Warfield (16) showed that if the venous pressure were kept constant, wide ranges of arterial pressure were without effect upon the excised heart of the frog and terrapin.

In the excised mammalian heart, Newell Martin (17) found that variations of arterial pressure within normal limits (25 to 140 mm. of mercury) were without influence upon the rate. These results were confirmed by Howell and Ely (18), who found that not only was the rate unchanged by changes in arterial pressure, but the relation between the duration of systole and diastole was unaffected. With very low pressures the rate may be slowed, due to insufficient nutrition of the heart. Hering (19), in his isolated heart-lung-carotid-jugular circulation, found no change or a slight increase in rate coincident with a rise of blood pressure. Magrath and Kennedy (20) found no constant changes in rate in the isolated cat's heart. Herlitzka (10), who has recently investigated the question in the isolated dog's heart fed by Locke's Solution, found that the size of contraction decreased as the pressure increased and increased as the pressure decreased. The rate increased with rise of pressure, but the change did not occur for some time and was apparently an indirect one, due to better nutrition of the heart from increased coronary flow. Both changes showed an optimum. With very high pressures (100 to 140 mm. of mercury) there was no change in rhythm, or even a decreased rate sometimes occurred. With very low pressures the rate was considerably reduced, due to poor coronary circulation. More recently still, Guthrie and Pike (21) have found enormous changes of rate with changes of arterial pressure in the excised organ. They reconcile the difference of their results with those of Martin and others by the assumption of some physiological controlling mechanism which causes the nerve-isolated heart *in situ* to behave differently toward changes of pressure from the excised organ. They believe this may be preserved in some excised hearts and not in others. They give no intimation as to the nature of this mechanism.



The weight of evidence tends, therefore, to support the view that in the heart isolated from all extrinsic nerve supply or excised from the animal, no changes of rate occur as a result of changes of arterial blood pressure that cannot be referred to nutritive conditions in consequence of changes of blood flow in the coronary arteries. Changes in blood pressure *per se* hence seem to be ineffective so far as changes of rate are concerned in the excised organ. Variations in rate with variations of arterial pressure have been more frequently observed in the nerve-isolated *in situ* heart than in the excised organ. Martin (17) believed this to be due to several causes. Ligation of the aorta in an animal leads to a more rapid filling of the auricle in diastole and probably increases the venous pressure. The temperature of the blood is, moreover, changed as a result of cutting off the warm blood from the intestines and abdominal organs. The blood probably also suffers unusual or excessive chemical changes. Tigerstedt ((3), page 299) repeats these factors which tend to reconcile the results obtained from the nerve-isolated *in situ* heart and the excised organ, and thinks that changes in rate in the former are especially due to the more rapid return of the blood to the venous end of the heart. Howell and Donaldson (22) found in the excised dog's heart variations in the pulse rate as a result of considerable variations in venous pressure. These variations were not always in the same direction.

The marked changes in rate that accompany changes in arterial blood pressure in the heart with full extrinsic nerve supply are thus largely if not entirely dependent upon the integrity of these nerves, of which the vagi are of paramount importance. After section of the vagi alone, increase of blood pressure may cause increase of rate (McWilliams (23)), or sometimes decrease and sometimes increase (Asp. (24), Nawrocki (6), and others). These changes in rate are always small.

In regard to the questions as to whether the changes that occur are due to a direct effect upon the cardio-inhibitory centre or whether this centre is affected through efferent nerves, the former view seems to be the one held by the majority of authors. The researches of François-Franck (25) indeed seem to prove conclusively that at least part of the effect is direct, the increased blood pressure stimulating the cardio-inhibitory centre. This investigator connected the cerebral ends of the carotids with a reservoir of defibrinated blood maintained at body temperature. The two vertebral arteries were ligated at their entrance into the skull and the cord cut below the bulb.

Rapid elevations of the pressure within the cerebral arteries caused marked slowing. How increased blood pressure stimulates the cardio-inhibitory centre is not known (see Biedl and Reiner (26)). Hill (27) supposed that a sudden and great rise of blood pressure might cause transitory brain anæmia by obliteration of the cerebral capillaries. Howell (28), however, showed this not to be true; the cerebral venous outflow under these circumstances was not reduced. It would seem that the vagus centre may be stimulated either by a considerable increase or a considerable decrease in its blood supply.

Contrary to the results of François-Franck cited above, Kochmann (29) in a recent paper finds that Ringer's Solution allowed to flow into the peripheral end of the carotid artery of dogs with intact vagi, under a pressure greater than the arterial pressure, produces no change or even a slight increase in the heart rate when the pressure is thus raised in the cerebral arteries. Slowing of the heart occurred in three of four experiments, however, when the pressure was raised toward the heart by directing the inflow into the central end of one common carotid artery. He does not state in the latter case whether the other carotid and the vertebrals were ligated in order to prevent the increased pressure from affecting the cerebral arteries.

Considering the slowing of the heart produced by a rise of blood pressure as due wholly or in part to a reflex with the vagi as the efferent path, there is no conclusive experimental evidence tending to show where the afferent path arises or through what nerves it is mediated. It has been generally assumed that it arises within the heart itself from nervous connections within the endocardium (Kochmann (29)). Hofmann (4) suggests the possibility of the phenomenon being a reflex one, the afferent path of which arises within the carotids, and cites the experiments of Pagano (30). Pagano observed, upon injection of hydrocyanic acid or nicotine into the common carotid artery of dogs after ligation of the internal carotids, a rise of blood pressure and slowing of the heart. This did not occur after removal of the two superior cervical and first thoracic ganglia. When the superior cervical and first thoracic ganglia of one side were removed, there was no such effect when the injection was made upon the operated side, while it occurred promptly if the injection was made upon the opposite (unoperated) side. From the other arteries and veins of the body investigated no such reflex effect upon the heart was observed. If the vagi were cut, a rise of blood pressure was observed following the

injection, but no change or even an increase of pulse rate occurred. Injection of salt solution into the peripheral end of the common carotids caused no decrease in heart rate after removal of the above-mentioned ganglia, while previously a slowing was observed when these structures were intact. This would tend to show that the reflex has its origin in the walls of the carotids. The author does not, however, state how often the negative result with pressure from salt solution after removal of the sympathetic ganglia was obtained, and leaves the impression that it was only in one experiment. In view of our experience we are convinced that the fact that a rise of pressure within the carotids and cerebral arteries (the internal carotids were apparently not ligated in this experiment) produced no effect upon the pulse rate after removal of the above-mentioned ganglia means little unless the observation were often repeated. It happens quite frequently that rises of pressure within the brain arteries, or pressure directed toward the heart, or both acting together, are without effect upon the heart rate late in experiments or with animals in bad condition, while previously such changes in pressure produced marked decrease in pulse rate. In many cases the first few increases of pressure may produce marked slowing, and the effect may become progressively less with each subsequent increase until the reaction finally disappears. This was especially noticeable in our experiments in which increase of pressure was produced in the cerebral arteries by the inflow of salt solution. The mechanism by which the slowing is brought about seems to be a delicate one and easily affected by abnormal conditions, such as a considerable addition to the total amount of blood. In raising the pressure before as well as after removal of the sympathetic ganglia in Pagano's experiment the brain and vagus centre were subjected to the increase of pressure. He apparently did not try the effect of increase of pressure confined to the carotids. The experiment of François-Franck cited above, in which cardiac slowing was obtained after section of the cord just below the medulla, speaks strongly against Pagano's view.

The depressor nerve has been considered as the possible afferent path of the reflex. Köster and Tschermak have shown, both on anatomical (31) and physiological (32) grounds, that this nerve is to be regarded as an afferent nerve of the aorta. They succeeded in demonstrating an action current in the depressor as a result of

increase of pressure in an isolated loop of the aorta. Ludwig and Cyon (33) have shown that stimulation of the central stump of this nerve may cause a decrease of pulse rate unaccompanied by change in blood pressure. Bayliss (34) observed that the decrease in heart rate from stimulation of the depressor disappeared after section of the vagi, and often an increased rate was observed under these circumstances. The brothers Cyon (35) in one instance in one experiment found that a rise of arterial pressure caused no effect upon pulse rate after section of the depressors, while previously it had caused a decrease. The experiments of Beidl and Reiner (36) and especially those of Stefani (37) have shown, however, that slowing of the heart accompanies increase of arterial pressure after as well as before section of these nerves. These results led these observers to conclude that such slowing is not of reflex origin, but due to a direct effect of the increase of blood pressure upon the cardio-inhibitory centre.

The presence of afferent fibres within the vagus in mammals which upon stimulation may cause reflex slowing of the heart was first shown by V. Bezold (38) and has been confirmed by numerous later observers. The slowing of the heart that results from increased arterial pressure, if due to a reflex, may well be entirely within these nerves, both afferent and efferent paths being fibres of the vagi. Brodie and Russell (39) have made a systematic examination of the different branches of the vagus in order to determine which fibres play the most important part in the reflex cardiac slowing which occurs from stimulation of this nerve. Central stimulation of the pulmonary fibres of the vagus gave the most marked decrease in heart rate. The cardiac branches were found to be much less effective, and the branches below the pulmonary fibres the least effective of all. Increase of arterial pressure (by nicotine, adrenalin, compression of abdominal aorta), however, caused as great a degree of slowing after section of the pulmonary fibres of the vagi as when these branches were intact. These results were obtained upon cats and dogs. The conclusion was, therefore, that reflex cardiac alteration in rate from increase of arterial pressure is due to excitation of the afferent cardiac fibres.

In view of the results from the literature, Tigerstedt (3) suggests the possibility that the phenomenon of decrease in pulse rate from rise of arterial pressure is due to two factors acting together, — a central effect upon the cardio-inhibitory centre, and a periph-

eral effect from rise of pressure within the heart or other part of the vascular system.

The investigations to be described in this paper were concerned in an attempt to determine whether the decrease in pulse rate that results from an increase of arterial blood pressure in an animal with intact vagi is due to a direct action of the increased pressure upon the cardio-inhibitory centre, or is peripheral in origin, arising from stimuli transmitted to the centre through afferent nerves. Furthermore, if found to be partially or wholly of reflex origin, we desired to determine, if possible, from what portion of the vascular system the afferent path arises and through what nerves it is mediated. The experiments performed were divided into a number of groups, and the methods used will be described in each section.

#### INCREASE OF BLOOD PRESSURE PRODUCED BY OCCLUSION OF DIFFERENT PORTIONS OF THE THORACIC AORTA.

For these experiments dogs were employed. The dogs were anæsthetized, placed upon artificial respiration, the internal mammary arteries ligated, and the chest opened. Ligatures were placed in the following situations: (*a*) around the ascending aorta as close as possible to the ventricle; (*b*) around the transverse arch of the aorta immediately beyond the origin of the left subclavian artery; (*c*) around the descending thoracic aorta a short distance above the diaphragm; (*d*) around the innominate and left subclavian arteries. Before placing the ligatures the regions were carefully dissected, especial care being used not to include branches of the vagi or other nerves. These ligatures were then passed through short pieces of glass tubing, and occlusion of the vessels was produced by traction upon them. The heart rate was recorded by air transmission. A marking-pen was employed to record the instant of occlusion and release of the vessels.

The changes in heart rate that resulted from occlusion of different portions of the aorta were as follows. Ligation of the descending aorta caused as a rule a considerable decrease in pulse rate. This decrease was often marked, in some cases the rate being reduced to as much as one half the normal. At other times the slowing of the pulse was of only moderate degree, and in certain cases, especially late in experiments or with animals who had suffered a considerable loss of blood, no changes of rate were observed.

Fig. 1 may serve as an example of the decrease in pulse rate that accompanies a rise of arterial pressure from occlusion of the descending aorta. (In this experiment the blood pressure was measured by a mercury manometer connected with the carotid, and occlusion of the aorta was produced in the unopened thorax by a method that will be described later.) Occlusion of the transverse aorta caused a similar decrease, although not, perhaps, to so great a degree as did ligation of the descending aorta. Occlusion of the ascending aorta gave, on the other hand, quite constantly no change or an increase in rate. Irregularities, due, as Knoll (40)<sup>2</sup> and others have shown, to extrasystoles of the ventricles, are not uncommon. In many cases, however, the rhythm is unaffected by such ligation, and the absence of a decrease in the heart rate is clearly evident in the records of experiments in which previous and subsequent occlusions of the descending or transverse aorta show a marked decrease in rate.

The failure of a decrease in the pulse rate to occur upon occlusion of the ascending aorta might be conceived to be due to the fact

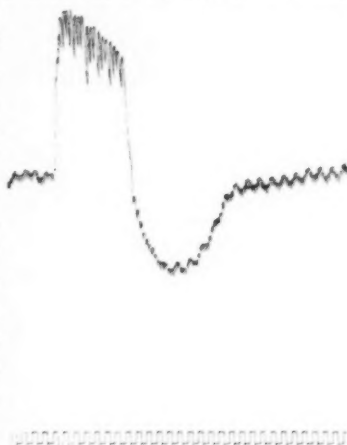


FIGURE 1. — Two thirds the original size. Decrease of heart rate from increase of blood pressure produced by ligation of thoracic aorta in a dog. Upper line, blood pressure and heart rate obtained with a mercury manometer connected with central end of left common carotid; middle line, time record (2 seconds intervals); lower line, zero blood pressure.

<sup>2</sup> Knoll has shown that if the pressure is raised in the left ventricle by clamping the aorta, arrhythmia occurs in this chamber, while if the pulmonary artery is occluded the irregularity occurs in the right ventricle. Since these experiments were performed, the paper of Pletnew (41) has appeared. In investigating arrhythmia from increase of intraventricular pressure obtained by ligation of the ascending aorta, this observer finds an increase of pulse rate following the occlusion. According to his observations, however, there is a transitory period of slight decrease in rate immediately following the occlusion which is present after section of the vagi as well as when the nerves are intact. We find no evidence of such transitory slowing in our records, and those published in connection with the article in question show at best only a slight indication of such decrease.



TABLE I.

No.	Procedure.	Heart rate in 30 seconds.			
		Before.	During.	After.	Change.
1	Ligation transverse aorta . . . . .	72	56	69	-16
2	Ligation descending aorta . . . . .	73	47	64	-26
3	Ligation transverse aorta . . . . .	69	43	62	-26
4	Ligation innominate and left subclavian arteries . . . . .	67	64	67	-3
5	Ligation transverse aorta followed by . . . . .	65	45	..	-20
	Ligation innominate and left subclavian arteries and . . . . .	..	45	..	0
	release of innominate and left subclavian arteries . . . . .	..	45	60	..
6	Ligation innominate and left subclavian arteries followed by . . . . .	66	60	..	-6
	Ligation transverse aorta, followed by . . . . .	..	48	..	-12
	release of innominate and left subclavian arteries . . . . .	..	33	56	-15
7	Ligation innominate, left subclavian and transverse aorta <sup>1</sup> . . . . .	62	37	57	-25
8	Ligation ascending aorta . . . . .	69	69	70	0
9	Ligation innominate, left subclavian and transverse aorta . . . . .	70	52	66	-18
10	Ligation transverse aorta followed by . . . . .	66	51	..	-15
	ligation of innominate and left subclavian arteries . . . . .	..	51	60	0
11	Ligation ascending aorta . . . . .	69	69	68	0
12	Ligation transverse aorta, left subclavian and innominate arteries <sup>1</sup> . . . . .	68	45	61	-23
13	Ligation ascending aorta . . . . .	61	65	66	+4
BOTH VAGI CUT.					
14	Ligation ascending aorta . . . . .	68	68	69	0
15	Ligation transverse aorta, left subclavian and innominate arteries <sup>1</sup> . . . . .	69	68	67	-1
16	Ligation transverse aorta . . . . .	67	66	66	-1
17	Ligation descending aorta . . . . .	66	69	67	+3
18	Ligation transverse aorta . . . . .	67	66	67	-1
19	Ligation left subclavian and innominate arteries . . . . .	67	67	69	0
20	Ligation ascending aorta . . . . .	69	68	68	-1
21	Ligation transverse aorta, left subclavian and innominate arteries <sup>2</sup> . . . . .	68	67	66	-1
<sup>1</sup> Ligation in the order named.		<sup>2</sup> All ligated at one time.			



that under these conditions the increased blood pressure did not affect the arteries supplying the cardio-inhibitory centre. The fall of pressure within these arteries as a result of the cutting off of this blood supply might likewise be conceived as the cause of the increased rate frequently observed. That this, however, was not the sole cause was shown by ligation of the transverse aorta before and after ligation of the innominate and subclavian arteries. In the former case, that in which the transverse aorta was first ligated, the subsequent ligation of the cerebral arteries did not cause a reduction in the slowing of the heart that was present as a result of the occlusion of the transverse aorta; that is to say, no increase in rate occurred upon relieving the cerebral vessels of the increased arterial pressure consequent upon ligation of the transverse aorta (No. 5 of Table I). Nos. 6, 7, and 9 of Table I are examples of occlusion of the transverse aorta following ligation of the innominate and left subclavian arteries. In these the increased blood pressure that resulted from occlusion of the transverse aorta did not affect the cerebral vessels and the vessels supplying the cardio-inhibitory centre. The result in such cases was a marked decrease in pulse rate. Furthermore, occlusion of the cerebral arteries alone in these experiments caused a slight decrease in pulse rate (No. 6 of Table I). In No. 6 of Table I release of the previously ligated cerebral arteries, the transverse aorta being still occluded, resulted in a further reduction of the rate which was present as a result of the combined occlusion of the cerebral arteries and aorta. The rate before any ligatures were tightened was 66 in thirty seconds. This was reduced to 60 by ligation of the cerebral arteries, and to 48 with the subsequent occlusion of the transverse aorta. With release of the cerebral arteries and the increased pressure allowed to affect the brain as well as the heart and aorta, a further reduction (to 33 beats per minute) occurred.

It therefore seems clear that, under the experimental conditions here described, an increase of pressure confined to the heart and thoracic aorta may cause a marked diminution in pulse rate. Examples such as No. 6 of Table I indicate that a rise of pressure within the cerebral arteries may also lead to the same result. When the ascending aorta is occluded, the increase of pressure is confined to the heart, and there is a marked fall of pressure in the thoracic aorta as well as in the cerebral arteries. The usual or at least frequent result was an increase in pulse rate. This increase was not due to

the anæmia of the brain, because ligation of the cerebral arteries alone in these experiments led to a slight decrease in rate. It was noted, furthermore, that increase of pulse rate upon ligation of the ascending aorta was rarely observed, and never to so great a degree after section of the vagi as when these nerves were intact.<sup>3</sup> The marked decrease in pulse rate that usually resulted from ligation of the transverse or descending aorta, and the absence of such change upon ligation of the ascending aorta,<sup>4</sup> indicated that increase of intracardiac pressure does not affect the cardio-inhibitory centre, and suggested that probably the thoracic aorta is the region to be considered as most important in this relation. These points were tested in the later experiments in connection with which they will be further considered.

After section of both vagi, occlusion of the descending or transverse aorta gave rise to a slight increase, a slight decrease, or no change in cardiac rate. A decrease was the most common result, but the change was always small, in no case amounting to over five or six beats per minute. Following the section of one vagus, either the right or left, the decrease in rate from occlusion of the transverse or descending aorta may occur as before or almost or quite disappear. This is in accordance with the general observation that one vagus usually carries the majority of the inhibitory fibres to the heart.

#### INCREASE OF PRESSURE CONFINED TO AN ISOLATED PORTION OF THE THORACIC AORTA.

The experiments reported above made it probable that increase of pressure within the thoracic aorta causes a decrease in pulse rate and that increase of pressure within the heart itself produces no such

<sup>3</sup> It might be supposed that if increase of pressure in the vessels supplying the cardio-inhibitory centre caused a slowing of the pulse rate, a decrease in pressure would lead to the opposite result. We have, however, when all the cerebral arteries are ligated, not merely a fall of pressure in these arteries, but an actual anæmia of the cardio-inhibitory centre, which, as is well known, is an efficient stimulus to this centre.

<sup>4</sup> Although great care was used in all experiments in which the ascending aorta was ligatured to avoid any branches of the vagi being caught in the ligature, the further precaution was taken to stimulate these nerves in the neck by a faradic current at a time when the ascending aortic ligature was tightened. The resulting inhibition of the heart showed that the ligature did not interfere with the action of the vagi.

effect. In order to investigate the former point more closely, the experiments described in this section were performed.

Dogs and rabbits were used for these experiments. The animals were anaesthetized, placed upon artificial respiration, the thorax opened, and ligatures placed around the ascending aorta, the upper part of the descending aorta, the left subclavian artery, and the superior and inferior vena cavæ. The innominate artery was closed with a bulldog clamp, and a cannula, connected with a reservoir of Ringer's Solution, was placed either within the innominate artery itself as close as possible to the aorta or in one common carotid facing toward the heart. In the latter case another cannula, attached to a short piece of rubber tubing and provided with a pinch cock, was placed in the other carotid facing toward the aorta. The reservoir of Ringer's Solution was kept in a warm bath at body temperature and connected with a cylinder of oxygen. To determine the pressure a mercury manometer with reading scale was connected with the reservoir. The procedure was as follows. With the Ringer's Solution under a pressure of from 160 to 280 mm. of mercury in the reservoir and connecting tube, but prevented from entering the aorta by a stopcock, the superior and inferior vena cavæ, the left subclavian artery, the ascending and descending aortæ were occluded in the order named. After a control period the stopcock was opened and the Ringer's Solution under the above-mentioned pressure was allowed to flow into the isolated loop of the aorta. After the desired interval of increased pressure the stopcock connecting with the Ringer's Solution was closed and the pressure in the loop relieved, either by opening the descending aorta, if the inflow cannula had been placed in the innominate, or by opening the outflow cannula in one common carotid when the inflow was through the carotid. The outflow from the loop was now closed, the stopcock connecting with the inflow cannula opened, and the pressure again raised in the loop. This could be repeated several times in succession.

Increase of pressure within an isolated loop of the aorta produced by this method caused in the majority of cases a decrease in rate, which returned to the previous rate or became even more rapid after the pressure was relieved. At times the effect upon the rate was marked, at other times the pressure thus produced was apparently without effect. The degree of trauma and shock consequent upon the extensive operative procedures required in these experiments, as well as the danger of injury to branches of the vagi and

other nerves by the dissection and ligatures, will, we believe, go far toward explaining the lack of constancy of our results. All of our experiments tend to show the remarkable susceptibility to abnormal conditions of the mechanism which causes a decrease in the pulse rate with increase of arterial pressure. This will be discussed later in more detail. We believe that the results of this series of experiments show that an increase of pressure, confined to the arch of the thoracic aorta in dogs and rabbits, may give rise to marked slowing of the heart.

Fig. 2 is a record from an experiment upon a dog. At 4 the two vena cavæ, the left subclavian artery, and the ascending and descend-

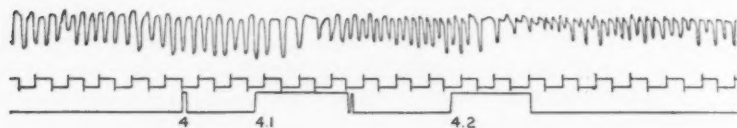


FIGURE 2.—Two thirds the original size. Decrease of heart rate produced by increase of pressure within an isolated portion of thoracic aorta. For description of record, see text.

ing aorta were ligated. At 4.1 the stopcock connecting with the reservoir of Ringer's Solution was opened, allowing the fluid to flow into the isolated portion of the aorta under a pressure of about 220 mm. of mercury. The pressure was continued as long as marking-pen line remained elevated. At the fall of this line the stopcock was closed and the pressure in the aorta relieved by opening the out-flow cannula connected with one common carotid. At 4.2 the out-flow cannula was closed and the pressure again raised in the aorta. The elevated portions of the lowest line thus indicate the time and duration of the periods of increased pressure.

The distance between the up strokes of the time-marking pen represents in this, as in the last record, intervals of two seconds. The occurrence or degree of slowing did not seem to differ when the inflow cannula was placed in one common carotid about midway of the neck instead of in the innominate artery approximately at its origin from the aorta.

INCREASE OF PRESSURE IN THE CEREBRAL ARTERIES BY INFLOW OF SALT SOLUTION UNDER A PRESSURE GREATER THAN THE BLOOD PRESSURE, AND ALSO INCREASE OF PRESSURE SIMILARLY PRODUCED TOWARD THE HEART.

Although the experiments of François-Franck (25) with artificial brain circulation seemed to be quite conclusive as to the effect of increase of blood pressure upon the cardio-inhibitory centre, the recent work of Kochmann (29) in which negative results were obtained led us to repeat the work of the latter. The method employed was similar to that used by Kochmann. A reservoir of Ringer's Solution under oxygen pressure was connected with cannulae placed in the two common carotid arteries, facing, one toward the brain, the other toward the heart. When the inflow was directed toward the heart, the left subclavian and the right vertebral arteries were ligated to prevent the pressure being transmitted to the cerebral arteries. In agreement with Kochmann, we found that pressure thus raised toward the heart may result in marked decrease of pulse rate. Contrary to his findings and in agreement with François-Franck, we found that a rise of pressure produced in the brain arteries may cause a marked retardation of the pulse or even a complete inhibition of short duration. A rise of pressure within the cerebral arteries seems to be even more effective in producing slowing than similar pressure exerted toward the heart. A record of an increase of pressure in the cerebral arteries is shown in Fig. 3. The pulse was recorded from the femoral artery by means of a Hürthle manometer. At the rise of the marking-pen line salt solution was allowed to flow into the peripheral ends of the two common carotids under a pressure of 200 mm. of mercury, and the pressure was continued as long as the marking-pen line remained elevated.

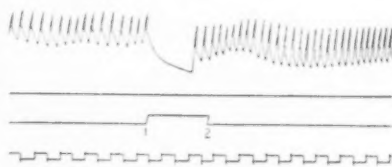


FIGURE 3.—One half the original size. Vagus inhibition produced by inflow of Ringer's Solution into cerebral arteries under a pressure greater than arterial pressure. Upper line, heart rate recorded with Hürthle manometer. Between 1 and 2 Ringer's Solution was allowed to flow into the peripheral ends of both common carotids under a pressure of about 220 mm. of mercury.

Mention has several times been made of the readiness with which the mechanism producing cardiac slowing under conditions of high blood pressure may fall out of function as a result of abnormal conditions to which the research animal is subjected. In certain cases, even without very extensive operative procedure, a change in pulse rate with variations of arterial pressure failed to occur. Not infrequently, indeed as a rule, in very long experiments in which much operating is done, the reaction may be present at the beginning to a marked degree, and subsequently may disappear entirely. In the experiments in which salt solution was allowed to flow into the carotids toward the heart or brain the reaction as a rule was marked in the first few trials, then became progressively less and finally completely disappeared. On the other hand in one experiment with a rabbit in which a quite extensive preliminary operative procedure had been carried out and a considerable loss of blood had occurred as the result of an accident, ligation of the descending aorta with a resulting considerable increase of blood pressure was without effect upon the heart rate. In this case, after about 200 c.c. of salt solution had been allowed to flow into the external jugular vein, the same procedure caused a marked decrease of pulse rate, and the animal continued to react to increased blood pressure over a considerable length of time. We have also succeeded in bringing back the reaction in several animals (dogs) by the hypodermic injection of one or two grains of morphia. In these animals the rapid heart rate which is invariably present when the reaction cannot be obtained, disappeared to a considerable extent, and increase of pressure then caused the usual marked slowing. In a number of cases in which increase of pressure failed to cause any slowing of the pulse, direct electrical stimulation of the vagus was also ineffective, showing these nerves to be out of function at the time. This, however, was not always true.

Of the two parts of the mechanism which tends to produce a slowing of the pulse with increase of pressure, namely, the direct effect of the increased pressure upon the cardio-inhibitory centre, and the stimulation of this centre through afferent nerves affected by the high pressure, as our experiments indicate, in the aorta, the former seems to be, so far as we can judge, the more effective and the one least harmed by abnormal conditions. We have had numerous examples of the disappearance of the reflex part of the mechanism while the direct effect upon the centre still remained. In such cases

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ligation of the descending aorta caused a decrease of heart rate only if the carotid and subclavian arteries were open and the pressure thus produced allowed to affect the cardio-inhibitory centre directly. In some of these cases increase of pressure confined to the heart and aorta by ligation of the cerebral arteries previous to ligation of the descending aorta, had formerly resulted in marked decrease in pulse rate. In other cases the effect of a rise of pressure when the cerebral

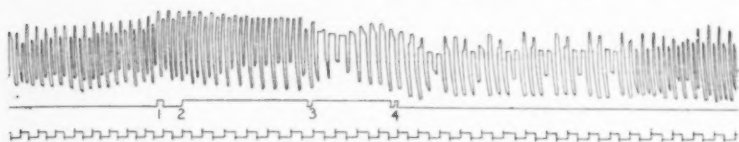


FIGURE 4. — Two fifths the original size. Showing partial disappearance of reflex slowing of heart from an increase of arterial pressure, the direct effect upon the cardio-inhibitory centre continuing to be marked. For description of record, see text.

circulation was excluded resulted in a slight but nevertheless perceptible slowing, and marked slowing occurred only when the increased pressure was allowed to affect the brain by release of the carotids and subclavians. An example of this is given in Fig. 4. At 1 in this tracing the left subclavian and innominate arteries were ligated and at 2 the descending aorta. The rate previous to ligation was 19.5 in ten seconds; during ligation of the cerebral arteries and aorta it was 18.5 in ten seconds. At 3 the cerebral arteries were released, the descending aorta remaining occluded and the increased pressure allowed to affect the brain. The rate fell to 10.5 in ten seconds (counted for six seconds). At 4 the descending aorta was released, the rate gradually increased and, twenty-four to twenty-six seconds later, had reached the rate present before ligation. Examples in which marked decrease in rate occurred in the first part of the above procedure when the increased pressure was confined to the aorta and heart are given in Nos. 6 and 7 of Table I.

#### INCREASE OF INTRAVENTRICULAR AND INTRACORONARY PRESSURE.

Dogs were employed for these experiments. The thorax was opened, a ligature was placed around the ascending aorta as close as possible to the heart, and a metal heart sound was passed down one common carotid artery until its end projected into the left ventricle. The heart sound was connected with a reservoir of Ringer's



Solution which was kept at body temperature and under oxygen pressure in the manner described in a previous section. After a control period the ligature around the aorta was tightly drawn around the sound, and salt solution allowed to flow into the ventricle under a pressure of from 160 to 280 mm. of mercury. The result of such an increase of pressure within the ventricle showed, in the great majority of cases, no decided or constant effect upon the heart rate. Frequently an increased rate resulted, often there was no change in

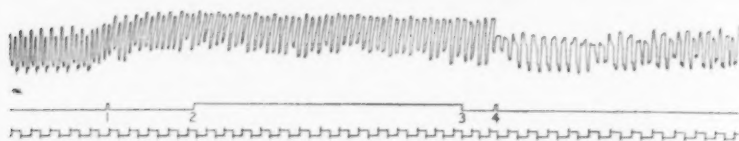


FIGURE 5.—Two fifths the original size. Comparison of effect of increase of intracoronary and of intra-aortic pressures upon the heart rate. For description of record, see text.

rate, and in a few instances slight slowing occurred, in no case approaching however the slowing obtained from increase of aortic pressure. The rhythm was often markedly affected, due to the production of extrasystoles. Increase of intracoronary pressure was obtained by a method similar to the above, except that the heart sound was introduced, not into the ventricle, but to a point in the aorta just above the semilunar valves. When the stopcock connecting with the reservoir of Ringer's Solution was opened, the coronaries could be seen to dilate and the whole organ enlarge. The results in the case of increase of intracoronary pressure were rather inconstant. Not infrequently slowing of the heart occurred, at other times no change in rate was present or the rate was increased. In the great majority of cases the decrease when present was small and by no means equal to that produced in the same animal by increase of aortic pressure. In the method employed a variable portion of the aorta was exposed to the increased pressure (that area included between the semilunar valves and the ligature around the ascending aorta), and slowing when it did occur might possibly have been due to this factor. If, following the increase of coronary pressure, the sound was withdrawn and the pressure raised in the aorta by the inflow of salt solution, the slowing of the pulse was usually pronounced, and always much greater than was ever present with increase of intracoronary or intraventricular pressure alone. An

example of this is given in Fig. 5. At 1 in this tracing the ligature occluding the aorta about the sound was tightened, and at 2 the inflow into the coronaries was begun. This was stopped at 3, the sound withdrawn beyond the ligature, and at 4 the fluid was allowed for a short time to flow into the aorta.

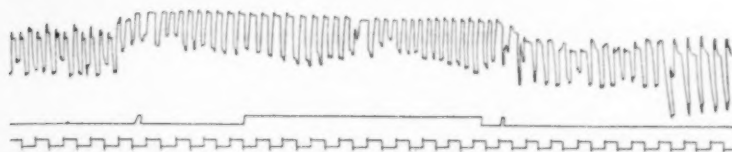


FIGURE 6.—Four sevenths the original size. Comparison of effect of increase in intra-cardiac and of intra-aortic pressures upon the heart rate.

Fig. 6 is the record of a similar procedure following increase of intraventricular pressure. The following table (Table II) gives the results of an experiment in which the pressure was raised in the

TABLE II.  
INCREASE OF INTRAVENTRICULAR AND INTRACORONARY PRESSURES.<sup>1</sup>

No.	Procedure.	Heart rate in ten seconds.		
		Before.	During.	After.
1	Ligation descending aorta . . . . .	22	16	20
2	Ligation descending aorta . . . . .	22	16	23
3	Increase of pressure in coronary arteries	22	22	23
4	Increase of pressure in coronary arteries	17	18	18
5	Increase of intraventricular pressure . .	19	20	20
6	Ligation descending aorta . . . . .	20	14	20
7	Ligation descending aorta . . . . .	25	25	25
8	Ligation descending aorta . . . . .	25	25	26

<sup>1</sup> In other experiments a reduction in rate of three beats in ten seconds has been observed in a few cases as the result of an increase of intracoronary pressure. The greatest reduction in the case of increase of intraventricular pressure was a single example of two beats in ten seconds (from fourteen to twelve). A closely following increase of pressure by occlusion of the descending aorta in this experiment gave rise to a decrease of ten beats in ten seconds (from twenty to ten).

heart and aorta by ligation of the descending aorta and in the ventricle alone and in the coronary vessels alone by the method above described.

While the experiments described in this section do not exclude an effect of increase of intracardiac and intracoronary pressure upon the pulse rate, they at least indicate that such an effect is of considerably less importance than an increase of pressure within the aorta.

#### THE AFFERENT PATH OF THE REFLEX.

One or more of three physiological systems of nerve fibres may be conceived of as forming the path of the afferent fibres which cause stimulation of the cardio-inhibitory centre as the result of increase

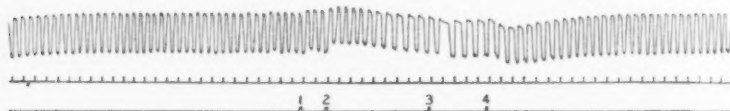


FIGURE 7.—Two fifths the original size. Reflex slowing of heart after section of accelerator nerves. For description of record, see text.

of arterial pressure; namely, the afferent fibres of the depressor, of the accelerator nerves of the heart and of the vagi. The experiments to be described in this section were undertaken with the purpose of determining which of these three is solely or in greater part concerned.

**The accelerators of the heart.**—The investigations to determine the part the accelerators play in this connection were performed upon dogs. The thorax was opened in the usual way, and ligatures placed around the innominate and left subclavian arteries and the descending aorta, beyond the origin of the latter vessel and above the point at which the aorta gives rise to the first spinal artery. The heart rate was recorded by air transmission. Occlusion of the innominate, left subclavian, and aorta in the order named caused a rise of arterial pressure confined to the heart and a portion of the thoracic aorta. Reflex slowing of the heart as a result of such an increase of pressure occurred after as well as before section of the accelerators.<sup>5</sup> The results from an experiment are given in Table III, and an example of reflex slowing after section of the accelerators in Fig. 7. At the first mark in this record the

<sup>5</sup> The whole superior thoracic ganglion of each side was removed, and the complete removal confirmed at autopsy.

TABLE III.

EFFECT OF INCREASED BLOOD PRESSURE AFTER SECTION OF ACCELERATOR NERVES.

Refer- ence to record.	Procedure.	Heart rate in 10 seconds.		
		Before.	During.	After.
ACCELERATOR NERVES INTACT.				
1	Ligation cerebral arteries and descending aorta . . . . .	24	20	23
ACCELERATOR NERVES CUT.				
2	Ligation cerebral arteries and descending aorta . . . . .	16.5	14	16
3	Ligation cerebral arteries and descending aorta . . . . .	17	15	16.5
4	Ligation cerebral arteries and descending aorta . . . . .	17	15	16
5 a	Ligation cerebral arteries followed by . . . .	16	16	
b	ligation descending aorta, . . . . .	..	14	
c	release cerebral arteries and . . . . .	..	11.5	
d	release aorta . . . . .	..	..	14-15
6 a	Ligation cerebral arteries followed by . . . .	15	16	
b	ligation descending aorta . . . . .	..	14-11	
c	release cerebral arteries and . . . . .	..	9	
d	release aorta . . . . .	..	..	13-15
9	Ligation cerebral arteries . . . . .	14.2	15	16
10	Ligation descending aorta . . . . .	14	7	14
12 a & b	Ligation cerebral arteries and descending aorta . . . . .	14	12	
c	release cerebral arteries and . . . . .	..	9	
d	release descending aorta. . . . .	..	..	13
14	Vagi cut . . . . .	..	..	16
15	Ligation descending aorta . . . . .	16	16	16

cerebral arteries were ligated, at the second mark the aorta, and at the third mark the cerebral arteries were released. The decrease in rate between the second and third marks represents the reflex slowing as a result of the rise of pressure in the aorta and heart. Following the third mark, the increased pressure affected also the cardio-inhibitory centre directly, resulting in a still further decrease in the rate. At the fourth mark the aorta was released. This record therefore shows the two factors acting to bring about a decrease in pulse rate with high arterial pressure, one reflex, the other direct, and also the fact that the former may occur independently of the accelerator nerves of the heart. Finally, it is to be mentioned that a number of experiments in this group gave negative results, probably due to the marked shock following upon section of the accelerators (see Hunt (42)).

**The depressor nerves of the heart.** — To investigate the importance of the depressors, rabbits were employed. The experiments of Biedl and Reiner (36) and those of Stefani (37), and the results we obtained from a like series of experiments performed at a time when we were unaware of the work of these investigators, showed that a decrease of pulse rate results in rabbits from increase of arterial pressure, after as well as before section of the depressors. They did not exclude, however, the possibility that the slowing under these circumstances might be entirely of central origin. In fact this result led the above-mentioned observers to conclude that the decrease of pulse rate from increase of arterial pressure is entirely due to the direct effect of the increased pressure upon the cardio-inhibitory centre. The depressors were apparently considered by them to be the sole possible afferent path for stimuli arising in the heart or aorta as a result of increased pressure, and since slowing of the heart occurred from such increase after section of these nerves, the slowing was believed to arise solely from a direct effect upon the centre. It was therefore of importance to determine whether or not the reflex slowing of the heart from increase of blood pressure is present after section of these nerves, or whether this part of the mechanism is abolished by such a procedure and the direct effect upon the cardio-inhibitory centre alone remains.

The procedure in the experiments was similar to that described above in the work on the accelerator nerves, except that the chest was not opened. The pulse rate was recorded by a Hürthle ma-

TABLE IV.

EFFECT OF INCREASED BLOOD PRESSURE AFTER SECTION OF DEPRESSOR NERVES.

Reference to record.	Procedure.	Heart rate in five seconds.		
		Before.	During.	After.
DEPRESSOR NERVES INTACT.				
1	Ligation descending aorta. . . . .	20	16	20
2 a	Ligation cerebral arteries followed by	21	21	..
b	ligation descending aorta . . . . .	..	12	22
DEPRESSOR NERVES CUT.				
3 a	Ligation cerebral arteries followed by	20	21	..
b	ligation descending aorta . . . . .	..	12	20.5
4 a	Repetition 3 a . . . . .	21	..	..
b	Repetition 3 b . . . . .	..	10	19
5 a	Repetition 3 a . . . . .	18	18	..
b	Repetition 3 b . . . . .	..	15	19
6 a	Repetition 3 a . . . . .	18	18	..
b	Repetition 3 b . . . . .	..	14	18
7 a	Repetition 3 a . . . . .	16	..	..
b	Repetition 3 b . . . . .	..	15	17
8 a	Repetition 3 a . . . . .	17	18	..
b	Repetition 3 b . . . . .	..	17.5	18
9	Ligation descending aorta . . . . .	18	16.5	..
10 a	Repetition 3 a . . . . .	15	15	..
b	Repetition 3 b . . . . .	..	15	16
11	Ligation descending aorta . . . . .	16	16	15

nometer connected with the left common carotid. In one experiment the same cannula led also to a mercury manometer, the tube leading to which being partially occluded so that the manometer recorded mean aortic pressure. The right common carotid below the origin of the right subclavian and the left subclavian below the origin of the vertebral artery were temporarily occluded by ligatures. The thoracic aorta was occluded by traction upon a ligature passed

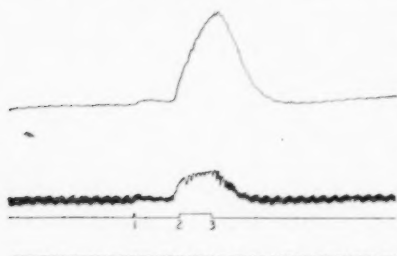


FIGURE 8.—About one-half the original size. Reflex slowing of heart after section of depressor nerves. For description of record, see text.

through the chest of the animal by means of a large aneurism needle and which entered and left close to the vertebral column, — a method of occlusion of the aorta with unopened thorax previously employed by Guthrie and Pike (43). The depressor nerves were sectioned in the neck. Reflex slowing of the heart from increase of aortic and ventricular pressure was obtained after

as well as before section of these nerves. Table IV gives the results from one of these experiments, and an example of reflex decrease in heart rate after section of the depressors is given in Fig. 8. The uppermost line of this record represents mean blood pressure obtained by partial occlusion of the tube leading from the arterial cannula to a mercury manometer. The second line from the top is the pulse rate recorded by a Hürthle manometer. The lowermost line records the time in one-second intervals. At 1 the cerebral arteries and at 2 the descending thoracic aorta were ligated. All vessels were released at the mark 3.

#### SUMMARY AND CONCLUSION.

The slowing of the heart that occurs in an animal with intact vagi from increase of arterial pressure is due to two factors which normally act together:

a. A direct effect of the increased blood pressure upon the cardio-inhibitory centre. This is shown in these experiments by the inflow of Ringer's Solution into the cerebral vessels under a pressure greater than the existing arterial pressure, and by the increased



blood pressure resulting from ligation of the aorta being allowed to affect the vessels of the centre by the release of the cerebral arteries which had been previously ligated.

*b.* A stimulation of the cardio-inhibitory centre through afferent nerves, resulting in a reflex slowing of the heart. This is shown in these experiments by the inflow of salt solution into the central (cardiac) end of one or both carotids with previous ligation of the vertebrals, by ligation of the aorta with previous ligation of the cerebral vessels, and by increase of pressure confined to an isolated portion of the thoracic aorta.

The afferent path of this reflex has its origin, at least to a great extent, in the thoracic aorta. Increase of pressure confined to this region caused a marked decrease of the pulse rate. Marked increase of intraventricular pressure produced by ligation of the ascending aorta and by inflow of salt solution under high pressure directly into the ventricle, with the exception of a few cases, caused no decrease in rate. Increase of pressure in the coronary arteries in some cases caused slowing, but never to the degree that the same increase of pressure in the aorta produced in the same animal. This was even more true of the decrease that was observed in some cases from increase of intraventricular pressure.

The reflex slowing of the heart from increase of arterial pressure persists in dogs after section of the cardiac accelerator nerves and in rabbits after section of the depressor nerves. It would seem, therefore, that the afferent path of this reflex is made up wholly or in part of fibres contained in the vagus nerves. These experiments do not prove that there are no such fibres in the depressors or accelerators, but only that they are not exclusively found in these nerves.

Evidence is brought forward to show that the reflex portion of the mechanism which causes decrease of pulse rate with increase of arterial pressure may disappear while the direct effect upon the cardio-inhibitory centre still remains.

Finally, it was found that this reaction of the organism is quite delicate and may be much reduced or abolished by abnormal conditions, such as those to which the research animal is necessarily subjected, especially when extensive operative procedure is required.

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## THE ANTAGONISTIC ACTION OF CALCIUM UPON THE INHIBITORY EFFECT OF MAGNESIUM.

BY S. J. MELTZER AND J. AUER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute  
for Medical Research.*]

### INTRODUCTORY.

CALCIUM and magnesium are chemically closely related bodies. They are also close companions in the fluid and solid tissues of the animal body. It seems to be a generally accepted opinion that both alkali earths exert influences in the animal body which run in a similar direction and are of a similar character. This frequently goes even so far as to assume that both ions are capable of substituting one another in the functions of the body. While there was a great deal of work done in recent years on the antagonism existing between the effects of calcium, sodium, and potassium (Ringer, Locke, Howell and his pupils, Jaques Loeb and his pupils, Overton), there was very little work done on the possible differences between the effects of calcium and magnesium, not to speak of an antagonism between them.

In speaking of the effects of calcium and magnesium we intend to deal here only with their relations to nerve and muscle. Here we encounter in the foreground the important and extensive work done by J. Loeb and his pupils. J. Loeb<sup>1</sup> put forward in 1899 the view that calcium and magnesium are ions which inhibit rhythmical muscular contractions. In studying the rhythmical twitchings of the frog gastrocnemius immersed in solutions of sodium chloride (and allied alkali compounds), Loeb discovered that these rhythmical contractions can be inhibited by the addition of compounds of po-

<sup>1</sup> J. LOEB in FICK's Festschrift, republished in "Studies in general physiology," Chicago, 1905, ii, p. 518.

tassium or calcium. It was then found further that "the entire group of (alkali earths) Be, Mg, Ba, Sr, and also Mn and Co" are capable of inhibiting these rhythmical contractions. Barium was later eliminated from this group, as it was found to cause contractions rather than to inhibit them. It should be stated that, in this first paper as well as in the many subsequent papers by Loeb himself and also by his many pupils, it was the inhibitory effect of calcium which was extensively studied and discussed. The possible inhibitory effect of magnesium upon muscular contractions was touched upon only occasionally and in a perfunctory way, while at the same time in the studies upon artificial parthenogenesis  $MgCl_2$  received foremost attention. However, in the recent writings of Loeb and his pupils the inhibitory effect of magnesium salts is brought out more prominently, although some<sup>2</sup> state expressly that "calcium inhibits more strongly than magnesium."<sup>3</sup>

Within the last few years we made several communications on the effects of magnesium salts.<sup>4</sup> They were all based on the hypothesis that magnesium favors inhibitory processes in the animal body. Some of the essential facts reported by us were that subcutaneous or intravenous injections of magnesium salts cause a state of anesthesia and paralysis from which, if the dose be not too large, the animal may completely recover.

The starting-point for our hypothesis was the observation, made several years before the first communication, that an intracerebral injection of two or three drops of magnesium sulphate caused a peculiar paralysis of the animal, while the injection of other salts was either indifferent or caused convulsions. This observation was

<sup>2</sup> BANCROFT: *The journal of biological chemistry*, 1907, iii, p. 209.

<sup>3</sup> The following variations of the same statement might perhaps serve as an illustration. In the first article (FICK's *Festschrift*, 1899) we read: "We are therefore indebted to the calcium contained in our blood for the fact that our muscles do not twitch continually" (quoted from "Studies in general physiology," part ii, p. 530, footnote). "It is due to the presence of Ca- (and K-) ions in our blood that our muscles do not contract rhythmically" (*This journal*, 1900, iii, p. 328). "As I stated six years ago, we owe it to the Ca- and Mg-salts in our blood that our skeletal muscles do not contract rhythmically like our heart" (*The dynamics of living matter*, 1906, p. 79).

<sup>4</sup> MELTZER and AUER: *This journal*, 1905, xiv, p. 366; 1906, xv, p. 387; 1906, xvi, p. 233; 1906-1907, xvii, p. 313. MELTZER: *Medical record*, 1905, lxviii, p. 965. MELTZER and HAUBOLD: *Journal of the American Medical Association*, 1906, xlvi, p. 647. MELTZER and AUER: *Journal of experimental medicine*, 1906, viii, p. 692.

demonstrated to the American Physiological Society at the New Haven meeting, 1899.<sup>5</sup>

While we are thus in full agreement with J. Loeb in regard to the inhibitory effect of magnesium, we could never persuade ourselves to an acceptance of the view that calcium also was a pure inhibitory factor. We are further not sure whether or not our definition of inhibition coincides with the one entertained by Loeb and his pupils. In our papers we variously took occasion to define the meaning of inhibition as we use it. It is an inhibition as exemplified by the vagus inhibition of the heart or the splanchnic inhibition of intestinal peristalsis. It is, then, not simply the prevention of a rhythmic movement, or the suppression of any vital process; it is the reduction or abolition of the irritability of the contractile tissues, including, of course, also the abolition of clonic or tonic contractions, that is, the production of a *relaxation* of the concerned muscular tissues. From this point of view we could never think, as Loeb's pupils do, of looking upon the reduction or suppression of diuresis, upon the suppression of glycosuria, or upon the prevention of hemolysis by calcium salts, as inhibitory phenomena. These suppressions might have been induced by some active, exciting, and not by an inhibitory process. Furthermore, it does not seem to us that the suppression of muscular twitching on a certain occasion is an all-sufficient fact for the consideration of calcium as a general inhibitory agent. It may still be an exciting agent at other times. Potassium, for instance, which, according to Loeb, suppresses the twitchings just as well as calcium, causes, according to Zoethout,<sup>6</sup> a pupil of Loeb, a tonic contraction of the frog's gastrocnemius muscle, which is surely an exciting process. Moreover, this tonic contraction caused by potassium can be inhibited by sodium chloride (Zoethout). Should we consider sodium chloride also an inhibitory agent?

<sup>5</sup> MELTZER: This journal, 1900, iii, Proceedings of the American Physiological Society. In our first communication on the effects of magnesium salts, where we have given some references to the literature on that subject, we inadvertently omitted to mention LOEB's view of the inhibitory effect of magnesium. We regret this oversight. It was caused by the fact that until that time LOEB himself discussed essentially and extensively the inhibitory effect of calcium, so that his casual brief references to magnesium entirely slipped our mind. We may mention here also that, according to LEE, *The microtometist's vademecum*, 4th ed., 1896, p. 16, TULLBERG (1892) and RADENBAUGH (1895) suggested the employment of magnesium salts for the anesthesia of certain invertebrate animals.

<sup>6</sup> ZOETHOUT: This journal, 1902, vii, p. 199.

Again, calcium itself decreases the irritability in one set of instances, in another set it unquestionably increases the irritability of muscle and nerve, and we do not need for this purpose to draw the mechanism of the heart beat into the discussion. The paralyzing effect of potassium, lithium, rubidium, and caesium salts upon muscle and nerve trunks can be promptly neutralized by the addition of calcium chloride (Overton<sup>7</sup>). The restoration of the lost irritability in these cases is surely a phenomenon just opposite to inhibition. Furthermore, the abolition of indirect irritability by sodium chloride (Carslaw,<sup>8</sup> Locke,<sup>9</sup> Cushing,<sup>10</sup>) and the "curare-like effects" of the salts of potassium, rubidium, ammonium, and caesium (Overton<sup>11</sup>), is promptly corrected by the addition of calcium. Here, again, calcium exerts a distinctly exciting and not an inhibitory effect upon the motor nerve endings.

In harmony with these facts was our own experience with calcium in its effect upon living mammals. We may mention it here briefly. Except for the inhibitory effect upon intestinal peristalsis (J. B. MacCallum) *we could never produce by subcutaneous or intravenous injections of calcium salts an anesthetic or paralytic effect in any way similar to that produced by magnesium salts. Even when large fatal doses were employed by intravenous injections, the animal was wide awake and the lid reflex, etc., was preserved until shortly before death.*

On the basis of all these facts we could not persuade ourselves that the various interesting and important phenomena observed by Loeb and his pupils upon the effects of calcium justified the positive assumption that calcium is an exclusively inhibitory agent. Moreover, we could not agree with the assumption, made by many others besides Loeb and his school, that calcium and magnesium are exerting a similar effect upon animal tissues. Our own studies<sup>12</sup> brought out various facts showing a striking contrast between the effects of these two chemical elements.

The continuation of the last-mentioned studies led up, finally, to the discovery that calcium is rather the strongest antagonist to the

<sup>7</sup> OVERTON: Archiv für die gesammte Physiologie, 1904, cv, p. 176.

<sup>8</sup> CARSLAW: Archiv für Physiologie, 1887, p. 429.

<sup>9</sup> LOCKE: Zentralblatt für Physiologie, 1894, viii, p. 166.

<sup>10</sup> CUSHING: This journal, 1902, vi, p. 77.

<sup>11</sup> OVERTON: *Loc. cit.*

<sup>12</sup> MELTZER and AUER: Journal of experimental medicine, 1908, x, p. 45.



inhibitory effects of magnesium. The report of these observations forms the subject of this paper.

Before entering upon the report we wish, however, to make the statement that Loeb<sup>13</sup> himself recently reported certain facts showing the existence of an antagonism between calcium and magnesium in their effects upon a jelly-fish (*Polyorchis*) found on the Pacific coast, and that Anne Moore,<sup>14</sup> a pupil of Loeb, reported several years before that the poisonous effect of a pure  $MgCl_2$  solution upon a fresh-water fish (trout) can be neutralized by the addition of  $CaCl_2$ .

Alfred G. Meyer<sup>15</sup> states that calcium assists sodium chloride in counteracting the inhibitory effect of magnesium which the latter exerts upon the movements of *Cassiopea*.

#### EXPERIMENTS ON THE ANTAGONISTIC ACTION OF CALCIUM TO THE INHIBITORY EFFECTS OF MAGNESIUM.

**Methods.** — The observations to be reported in this paper were made essentially on rabbits. The solutions of magnesium salts were given either subcutaneously or intravenously; the calcium salts were given in all cases intravenously, in some cases through the ear vein, in others through a cannula inserted into one of the external jugular veins. Of the various magnesium salts there were used: the sulphate, chloride, nitrate, and acetate; of the calcium salts: the chloride, nitrate, and acetate. For the subcutaneous injection of magnesium,  $m/1$  concentrations were used in all cases. In the intravenous experiments the magnesium salts were used in various concentrations, mostly in  $m/8$  solutions. The calcium salts in all cases were  $m/8$ . The influence of these salts upon respiration, blood pressure, cardiac vagus, etc., were studied in numerous experiments by the graphic method. Also stimulations of the peripheral and central ends of a sciatic nerve were made for the purpose of studying the antagonistic effects under discussion upon the motor and sensory functions.

<sup>13</sup> LOEB: *Journal of biological chemistry*, 1905-1906, i, p. 427.

<sup>14</sup> ANNE MOORE: *This journal*, 1901, iv, p. 391.

<sup>15</sup> ALFRED G. MEYER: *Rhythmical pulsation in Scypho-medusæ*, Carnegie Institution of Washington, 1906, p. 46.

In all experiments which required cutting, ether was used freely during the time of operation.

**Experiments with subcutaneous injections of magnesium salts.** — In our first paper on general anesthesia due to magnesium salts, we stated that for magnesium sulphate in subcutaneous injections the anesthetic dose is between 1.25 and 1.75 gm. per kilo. (The water of crystallization is included in all our figures.) Doses exceeding 1.75 per kilo were usually fatal. For  $MgCl_2$  the dose was even lower. With an anesthetic dose, thirty to forty minutes after a subcutaneous injection, the animal would usually lie perfectly limp without any lid reflex and without a reaction to a stimulation. When the dose exceeded the safety point, the respiration would cease pretty early after the injection, the cardiac activity usually persisting longer than the respiration.

In our present series we usually employed fatal doses of the magnesium salts, and calcium was injected at various stages after the onset of a pronounced effect. We shall illustrate our results with a few abbreviated protocols.

*Experiment 1.* — Gray male rabbit, 1550 gm.

10 A. M. Injected subcutaneously, middle of abdomen, 13 c.c.  $MgCl_2$  in  $m/1$  solution = about 2 gm. per kilo.

10.30. Respiration very shallow, slow, practically no lid reflex, limp. Injected through ear vein 8 c.c.  $CaCl_2$  in  $m/8$  solution. Respiration at once deepened and quickened, animal turned over and sat up.

10.50. Again under the influence of magnesium, lying on side, but respiration good. Injected again 6 c.c. of  $CaCl_2$   $m/8$  through ear vein. Animal turns over, sits up, and remains well.

Two grams per kilo of  $MgCl_2$  is a surely fatal dose; the animal was completely paralyzed and already very near a fatal issue. The injection of  $CaCl_2$  immediately improved the respiration and reversed the paralysis.

*Experiment 2.* — Gray female rabbit, 1210 gm.

11.10. Injected subcutaneously in the right side of abdomen 10 c.c.  $MgCl_2$  in  $m/1$  solution; slight loss; added 2 c.c. of the solution; altogether more than 2 gm. per kilo of  $MgCl_2$ .

11.40. Lid reflex gone, respiration slow and shallow, completely relaxed, no response on pinching tail or leg.

Injected through ear vein 6 c.c.  $m/8$   $\text{CaCl}_2$ . After 2 c.c. respiration deepened and quickened; after finishing the injection lid reflex active and animal turns over and sits up.

12.10. No lid reflex again, respiration shallow. Again given through ear vein 6 c.c.  $m/8$   $\text{CaCl}_2$ . Same effect as before, animal sits up and remains normal.

*Experiment 3.*—White rabbit, 1450 gm. Animal on holder, ether given, and a cannula inserted into the left jugular vein.

10.15. Injected subcutaneously 14.5 c.c.  $\text{MgSO}_4$  in  $m/1$  solution = 2.5 gm. per kilo.

10.35. Respiration rapid and shallow, lid reflex reduced.

10.42. Respiration shallow and labored, legs limp, lid reflex slight.

10.44. Started calcium acetate, running slowly from burette into external jugular vein. Respiration improved at once, is deeper and more rapid, *hind legs become stiff*.

10.50. 10 c.c. ran in. Lid reflex active, respiration good. Strong struggle on pinching toes.

12.00. Respiration and heart good, but lid reflex reduced again and only moderate motion on pinching tail.

1.15. Breathes easily, moves occasionally with some vigor.

3.00. Wound sewed-up and animal taken from table. No further trouble.

On account of the large dose of  $\text{MgSO}_4$  and the occasional failure of the ear vein method, when it is most urgently needed, a cannula was inserted into the external jugular vein. Although the dose of  $\text{MgSO}_4$  in this experiment was quite large, the single dose of 10 c.c.  $m/8$  calcium acetate was sufficient to bring about complete recovery of the animal without sequela. A noteworthy incident is *the stiffness of the legs after the introduction of  $\text{CaCl}_2$ , a sharp contrast to the paralysis just a few minutes before*.

*Experiment 4.*—White and black male rabbit, 1070 gm. Animal on holder, ether given, and cannula inserted into the external jugular vein.

12.05. Injected subcutaneously in the epigastric region 13 c.c.  $\text{MgSO}_4$  in  $m/1$  solution = 3 gm. per kilo.

12.38. Lid reflex nearly gone, breathing very shallow, some slight, short twitchings (asphyxia?).

12.40. Lid reflex completely gone, animal completely paralyzed, no response on pinching tail, respiration very shallow.

12.45. Respiration hardly perceptible, heart good. Started  $m/8$

$\text{Ca}(\text{CH}_3\text{COO})_2$  from burette very slowly. Respiration immediately deeper and more rapid.

12.47. 2 c.c. of the calcium acetate ran in so far. Lid reflex returned, sensation present, struggled twice.

12.50. Legs stiff, left extended, right flexed, but resistant to motion.

12.55. 10 c.c. in; stopped the infusion.

1.05. Respiration, heart, lid reflex good. Animal attempts to free itself.

2.45. Respiration, heart, sensation good, lid reflex less prompt. Given again 4 c.c. calcium acetate and animal taken from board; uneventful recovery.

In this experiment the rabbit recovered from a dose of 3 gms.  $\text{MgSO}_4$  per kilo, and again the one dose of 10 c.c. calcium acetate was practically sufficient to bring about permanent recovery; and here again there was stiffness of the legs after the use of calcium acetate.

*Experiment 5.* — White male rabbit, 1530 gm.

11.05. Injected subcutaneously 12.5 c.c. magnesium nitrate in  $m/1$  solution = 2 gm. per kilo.

11.25. Lying on side, slow and shallow respiration, no lid reflex.

Injected through ear vein 7 c.c. calcium nitrate in  $m/8$  solution. Immediately after finishing injection animal sat up and moved around. Respiration excellent.

11.45. Lying on side again, no response to pinching tail or leg, respiration shallow, and no lid reflex. 1 c.c. calcium nitrate given through ear vein. Animal sat up.

11.55. Lying on side again. Attempt made to give again calcium nitrate by ear vein, but was unsuccessful.

2.05. Lying on side still, urinated a good deal; respiration slow, but fairly deep. Responds with movements of extremities to pinch of tail. (Animal found dead next morning, death apparently being due to perforation of the colon produced in an attempt to wash it out.)

Experiments like the above were made in large number and practically without a single failure. As long as there were some efficient heart beats and a few gasps of respiration, the intravenous injection of a solution of calcium chloride infallibly improved the respiration instantaneously and revived the animal. As can be seen from

the above protocols, with the assistance of the calcium injections animals survived even such large fatal doses as 3 gm. per kilo of a magnesium salt. When the quantity of the subcutaneously injected magnesium salt exceeded the fatal dose perceptibly, frequently a single injection of a calcium salt would not be sufficient to establish the recovery permanently. After a sudden and apparently complete awakening from the deadly influence of the magnesium, the animal would gradually sink for a second time into a comatose state. A second injection would again completely reawaken it. In most cases such a second injection was apparently unnecessary. If for some reason the second injection was not made, the animal, as a rule, finally recovered anyhow. Without the second injection the animal would lie in an anesthetic and relaxed state for hours, but the respiration seemed not to be in danger. Here was the significant difference between the anesthetic state before the first injection of the calcium and the similar state which set in again after this injection: that the first primary state was progressive, full of immediate danger for the respiration, while the second state was at the worst stationary, and with a tendency to regression, the respiration being at no time in real danger.

The reason for the relapse into the secondary state seems to be simply this: the intravenously injected calcium is capable of neutralizing of course only that part of the magnesium which it finds already absorbed from the subcutaneous tissues. But since the magnesium continues to be absorbed after the injection of the calcium, especially when the injected magnesium dose was large, a new state of anesthesia is liable to come on. However, the effect of the magnesium upon the respiratory centre seems to be easily kept in check even by a very small dose of calcium, and such a small dose seems to remain active for some time after an intravenous injection of that substance, as will be seen in experiments to be described later. Hence the favorable condition in the secondary state of anesthesia which sets in after an injection of calcium.

A large dose of calcium is sometimes sufficient to neutralize efficiently all the depressing effects of the subsequently absorbed magnesium, as can be seen from Experiments 3 and 4, in which a single injection of 10 c.c. calcium was sufficient to overcome a magnesium dose of 2.5 or 3 gm. per kilo.

From these considerations it follows that the later the injection of calcium is given, the better the chance of neutralizing a larger

part of the absorbed magnesium. On the other hand, we have to record the fact that the danger of respiratory paralysis can be obviated by an injection of only a small quantity of a calcium salt, as we have learned in experiments which were prepared for a demonstration or for photographing. At the approach of danger an injection of 1 c.c. of calcium will keep the animal out of danger for some time, while it will remain in a complete state of anesthesia. Fig. 1 illustrates the last-mentioned condition. The animal received

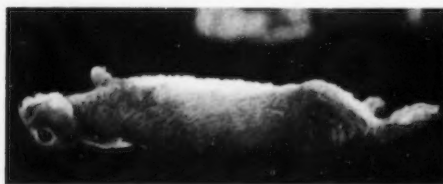


FIGURE 1. — Rabbit under the influence of 2 gm.  $MgCl_2$  (crystals) per kilo body weight, given subcutaneously in  $m/1$  solution. In order to prevent respiratory paralysis from this lethal dose 1 c.c. of  $m/8$   $CaCl_2$  had been injected into the ear vein. Note complete relaxation of animal.

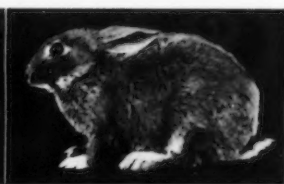


FIGURE 2. — Same animal less than one minute after an intravenous injection (ear vein) of 6 c.c.  $m/8$   $CaCl_2$ . Animal able to move about easily.

$MgCl_2$  subcutaneously 2 gm. per kilo for the purpose of having it photographed before and after the injection of calcium. However, twenty-five minutes after the injection the animal was very comatose, bordering closely on the danger line, while the arrangements for photographing were not yet ready. The animal received then 1 c.c. of  $CaCl_2$   $m/8$  through the ear vein. It remained then for over half an hour in a stationary state of deep anesthesia, at the end of which time it was photographed. The picture shows the complete relaxation of the entire animal, which could be placed in any position; it shows also the clamp upon the ear margin, where the injection was given. Fig. 2 shows the same animal about one minute after an injection of 6 c.c. of  $CaCl_2$  in the marginal vein of the same ear in which the first injection was given.

When the quantity of magnesium injected was not much more than the maximum anesthetic dose, an intravenous injection of a few c.c. of a calcium salt in  $m/8$  solution restored the animal permanently to a normal state. A number of rabbits received, at intervals of one or two days, several injections of magnesium and calcium salts without any noticeable detriment.

We shall cite here one more protocol of an experiment on a monkey.

*Experiment 6.* — Female monkey (*Macacus rhesus*), 2200 gm.

12.00 M. Injected subcutaneously, front of thorax, 9 c.c. of  $\text{MgSO}_4$  in  $m/1$  solution = 1 gm. per kilo, and returned animal to the cage.

1.00. Found perfectly limp in cage, only occasional very shallow respiration; no lid reflex; heart beating well. A cannula was hurriedly introduced into the right femoral vein and 2 c.c. calcium acetate in  $m/8$  solution injected. At once respiration became deep and rapid, lid reflex active; moved legs. Animal remains on holder.

1.40. Respiration fair, but with active expiration; does not follow objects with the eyes.

1.42. Injected again through femoral vein 2 c.c.  $m/8$  calcium acetate. Respiration at once deepened, became more rapid, and less expiratory in character; lid reflex active; no change in heart rate. Follows movements of people with the eyes, moves head; no movements of arms and legs. Remained in about the same condition until about 4.30, when she began moving arms and legs.

4.55. Shows fight now, opens mouth when approached.

5.30. Wound sewed up, attempts to escape when removed from holder. Sits up in cage, moves about; completely recovered.

The injection was given probably into the pectoral muscle, or at least subfascially, as in purely subcutaneous injections 1 gm. per kilo has never such a profound effect upon monkeys. The neutralizing effect of the calcium injection was as striking here as in rabbits.

In the foregoing experiments we learned that *the intravenous injection of any calcium salt is capable of neutralizing nearly instantaneously all the symptoms produced by the subcutaneous injection of any magnesium salt.* We hardly need to state expressly that the intravenous injection of calcium will not neutralize all doses of magnesium. We did not go further in the subcutaneous injections of magnesium salts than 4 gm. per kilo. In this case the calcium injections relieved temporarily the symptoms, but the heart became irregular and failed ultimately, after a good deal of calcium was given.

**Experiments with intravenous injections of magnesium.** — We shall now relate some of our observations made in experiments in which both salts were given slowly intravenously.



*Experiment 7.*—White female rabbit, 2650 gm. Ether; cannulas introduced into both external jugular veins and connected with burettes, one containing  $\text{MgCl}_2$  in  $m/2$  solution and the other containing  $\text{CaCl}_2$  in  $m/8$  solution; tracheotomy; a pleural cannula introduced into the left pleural cavity and left lung redistended; cannula connected with a Marey tambour to record the respiration.

After the animal recovered sufficiently from the ether,  $\text{MgCl}_2$  was run into the jugular vein fairly slowly, about 1 c.c. in two minutes. After nine minutes, when about  $4\frac{1}{2}$  c.c. had been infused, the animal became asphyxiated and artificial respiration was started. The infusion of  $\text{MgCl}_2$  continued. About a minute later the artificial respiration was discontinued for twenty-four seconds and no sign of spontaneous respiration appeared, the writing-lever marking only the heart beats. The artificial respiration was resumed. It was discontinued again after forty-five seconds, and thirty-two seconds later, after no sign of a spontaneous respiration had appeared, the infusion of  $m/8$   $\text{CaCl}_2$  into the other jugular vein began, while the infusion of  $m/2$   $\text{MgCl}$  still continued. A few seconds later the first spontaneous respiration appeared, and within twenty seconds, when not much more than half a cubic centimetre of the calcium solution had entered, the respiration was quite normal again. (The tracings in Fig. 3 illustrate the essential points of the above statement.)

This experiment demonstrates that a minute quantity of a dilute solution of calcium chloride is sufficient to practically instantaneously restore the respiration inhibited by 7 c.c. of  $m/2$  magnesium chloride. However, we have to add that in this instance after the first appearance of asphyxia less than 2 c.c. magnesium was given and less than four minutes' time passed before the infusion of the solution of the calcium salt was begun. The neutralizing effect of the calcium loses its promptness in proportion with the increase of the quantity of magnesium salts infused and with the prolongation of the interval passing between the appearance of asphyxia and the beginning of infusion of the calcium solution, so that a condition may finally present itself when the administration of calcium may remain with very little or even with no effect.

*Experiment 8.*—Black female rabbit, 1480 gm. Ether; cannulas in both external jugular veins; tracheotomy; left sciatic exposed, intact, placed in a Ludwig electrode and connected with an induction coil (Porter); ether discontinued.

12.05. Start infusion of  $m/8$   $MgCl_2$  into left jugular vein. Stimulation of sciatic with 100-coil distance gives a strong contraction of the left leg as well as a general reaction.

12.13. 17 c.c. ran in, lid reflex gone, respiration very shallow.

12.14. Practically no respiration, starting artificial respiration.

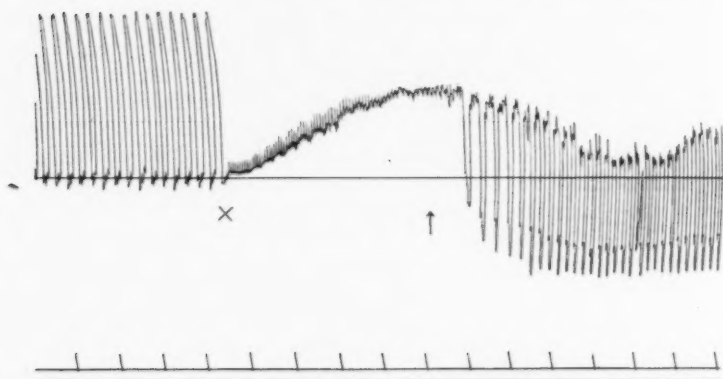


FIGURE 3.—Respiratory tracing taken by means of a cannula from the pleural cavity. The horizontal line cutting the tracing marks atmospheric pressure: above the line is positive pressure, below the line is negative pressure. Time, six-second intervals. The beginning of the curve shows artificial respiration, spontaneous respiration having been abolished by  $m/2$   $MgCl_2$  still flowing in; at the point marked X artificial respiration was discontinued, and the curve now shows volume changes due to the heart beats. For over thirty seconds no spontaneous respirations appeared; then (at the arrow-mark)  $m/8$   $CaCl_2$  was infused slowly into the jugular vein,  $m/2$   $MgCl_2$  still running into the other jugular vein at the original rate. After less than six seconds spontaneous respirations appeared in normal strength and rate (the down stroke in this part of the curve marks inspiration); and negative pressure was largely re-established in the pleural cavity after less than 1 c.c. of Ca had entered the circulation.

12.15. 21 c.c. infused. Sciatic 100: left toes move, also right reflexly, but no pain.

12.17. Sciatic 100: very slight contraction of left toes, no reflex to right.

12.21. Began infusion of  $CaCl_2$   $m/8$  into right jugular vein; 4 c.c. ran in fairly rapidly. ( $MgCl_2$  continued.) Respiration began at once. Sciatic 100: good contraction of left hind leg, strong reflex to right hind leg and also to front legs.

In this experiment spontaneous respiration was abolished by the magnesium solution long before a great reduction of irritability of the sciatic nerve to electrical stimulation was obtained, and here again

the irritability for afferent impulses was affected earlier than for motor impulses. Both, however, were reduced nearly to zero, at least for the original strength of stimulus, and the irritability of both recovered fairly well soon after the infusion of 4 c.c.  $m/8$   $\text{CaCl}_2$ .

In some of the experiments of the kind last quoted, after the first recovery of the animal, the experiment was repeated by again bringing the animal under the influence of magnesium, and attempting to attain at least a temporary recovery by a second infusion of a calcium solution. In these instances it was often noticed that it now took a much larger dose of the magnesium salt to completely abolish respiration than on first trial, and that now the motor and sensory functions, as tested by stimulations of the sciatic nerve, succumb to the influence of magnesium more readily than the respiratory mechanism, — a condition which is the reverse of that which usually takes place at the primary experiment. *Apparently the respiratory mechanism, which becomes affected by magnesium more readily than any other function, is also more easily protected against this influence by calcium.* In the secondary experiments, therefore, the remainder of the calcium (within the blood or the tissues?) from the previous infusion is still able to keep up the resistance of the respiratory mechanism against the renewed attack of the magnesium.

The behavior of the motor function of the sciatic nerve with reference to the reversing effect of calcium was not as simple and uniform as that of the other functions, for instance as that of the lid reflex or the respiration. In the first place the degree and the rapidity of recovery was variable. While it was prompt and complete in one case, it was slow and insufficient in other cases. In two instances there was practically no recovery. However, in these cases also the calcium infusion evidently stopped the progress of the paralysis, although the infusion of the magnesium solution still continued. It must be stated, further, that in these cases the sciatic nerve was cut, and we thought it possible that, on account of the vaso-dilatation hereby produced in the muscles of the leg, more magnesium accumulated there and the inhibitory effect was therefore more profound; against this the incoming calcium was less efficient than against the inhibitory effect upon the other organs which harbored a smaller quantity of magnesium.

It should be remembered, however, that this unsatisfactory behavior of the motor apparatus of the leg applies only to the artificial conditions as we create them by stimulating the motor nerve electri-

cally. The normal motor impulses of the leg show the same prompt reversibility as the other functions. An animal which was completely paralyzed by magnesium, on receiving calcium, struggles with the legs as readily and as promptly as it breathes and winks.

We append the following greatly abbreviated protocol for the purpose of showing the inefficiency of calcium after the previous administration of a large dose of magnesium.

*Experiment 9.* — Gray rabbit, 1490 gm. (prepared as in Experiment 8, except that a Petzold induction coil was used). Sciatic 110: moderate general effect (motor, reflex, pain).

10.56. Began infusion of  $m/8$   $MgCl_2$ .

11.01. 21 c.c.; respiration practically nil; started artificial respiration.

11.07. 38 c.c. ran in (more than 3 c.c. a minute!).

11.08. Sciatic 110: no effect whatsoever. (Slight lid reflex present?!)

11.10. 46 c.c.  $m/8$   $MgCl_2$ . Started calcium acetate  $m/8$ .

11.11. 6 c.c. calcium acetate in; no respiration.

11.13. 49 c.c. magnesium and 19 c.c. calcium. No respiration and sciatic 110 negative. Stopped magnesium.

11.15. 30 c.c. calcium acetate ran in. No respiration; heart stopped beating, no recovery.

The infusion of calcium in this case had not the slightest effect; it perhaps even hastened the death of the animal. Here 46 c.c. of  $m/8$   $MgCl_2$  were infused before calcium was started. In other instances in which 33 or 35 c.c. of a magnesium solution ran in before calcium was given, a final recovery was obtained, but its onset was late and it progressed slowly. This simply means that not all quantities of magnesium and not all stages of its poisonous effects can be overcome by calcium, — a fact to be expected *a priori*.

*Experiment 10.* — White male rabbit, 2200 gm. Ether; cannulas into both jugular veins and in carotid artery, the latter connected with a mercury manometer; a saturated solution of sodium sulphate used as connecting fluid; left vagus cut; Petzold coil with two Daniell cells used; the left burette filled with  $MgCl_2$  in  $m/2$  solution and the right burette with  $CaCl_2$  in  $m/8$  solution.

The blood pressure was about 125 mm. The secondary coil at 85 mm. distance was the minimal stimulus for the peripheral end of the vagus which stopped the heart (Fig. 4a).

The infusion of  $m/2$   $MgCl_2$  began, and continued very slowly,

about 1 c.c. in eighty seconds. The blood pressure very soon began to fall slightly, but continuously. When 2 c.c. of the magnesium solution was in, stimulation with 85-coil distance did not completely stop the heart. When a little over  $2\frac{1}{2}$  c.c. was infused, asphyxia set in and artificial respiration was started. Soon after, when about 3 c.c. of the solution had run in, 85-coil distance caused only moderate slowing of the heart and slight fall of pressure. Still later, after 4 c.c., even 50-coil distance had no effect. After 6 c.c. 40-coil distance failed to stop the heart. Now, while the magnesium solution continued to run in, the infusion of  $m/8$   $\text{CaCl}_2$  began. The blood pressure, which had sunk to about 40 mm., immediately began to rise, and about fifty seconds later not only a stimulation with the secondary coil at 40, but even with a coil distance of 100, stopped the heart completely; and even with a coil distance of 120 mm. there was a distinct effect upon the heart beat. The blood pressure rose to about 70 mm. after only 1 c.c. of the calcium solution (see Fig. 4 b).

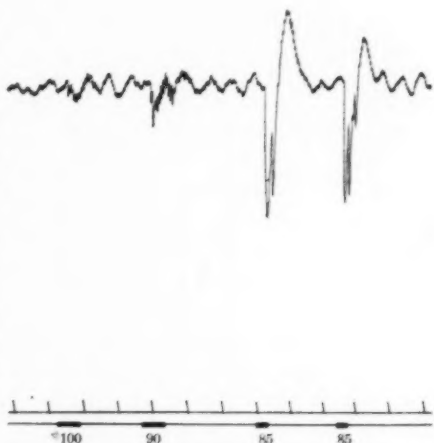


FIGURE 4 a. — Five sixths the original size. Normal blood-pressure tracing from the carotid artery of a rabbit showing minimal effective stimulus for the left peripheral vagus. Time in six-second intervals; this line also marks atmospheric pressure; the lowest line shows the duration of stimulation.

In this experiment we learn, first, that magnesium salts in concentrated solution reduce the cardio-inhibitory effect of the vagus. We learn further that this depression of the irritability of the vagus can be promptly removed by a relatively small dose of calcium and, what is more, the irritability becomes stronger than it was before the use of the magnesium. Before the magnesium was started, the heart was stopped by a stimulation of the vagus, when the secondary coil was at a distance of 85 mm. When it was at 90, it had practically no effect. After some calcium was infused, not only at 90 and at

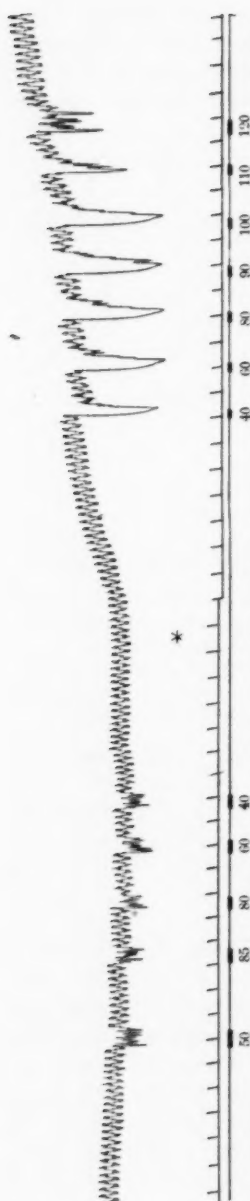


FIGURE 4A.—Blood-pressure tracing from the same animal (Figure 4C) under the influence of  $m/2$   $MgCl_2$ . Artificial respiration. The lowest line marks the duration of stimulation. Time in six-second intervals; the time line also records the atmospheric pressure. At \*  $m/8$   $CaCl_2$  was injected slowly into jugular vein,  $m/2$   $MgCl_2$  still being infused into the other jugular vein at the original rate. Note the rise in blood pressure following immediately upon the entrance of  $CaCl_2$  into the circulation, and the greatly increased effectiveness of left peripheral vagus stimulation. Before the injection of calcium, stimulation with the secondary coil at 40 produced only a slight effect; after the calcium, stimulation with the coil at 120 showed a definite fall in blood pressure.

100 did a stimulation of the vagus completely stop the heart, but also even at 120-coil distance the stimulation of the peripheral end of the vagus had a distinct cardio-inhibitory effect. This means that the calcium not only reversed the depressing effect of the magnesium, but increased the irritability of the vagus beyond its original normal threshold. Finally, calcium caused also an immediate rise of the blood pressure, which was greatly reduced by the magnesium. It should be remembered that all these neutralizing effects of the calcium were brought out while the magnesium was still running in at the original rate.

#### DISCUSSION.

The experiments communicated in this paper, which form only a small fraction of those performed, established that the intravenous infusion of various calcium salts is capable of completely reversing the pronounced inhibitory effects brought on by the various magnesium salts. The respiratory paralysis, the lost lid reflex, the motor paralysis, the lost general reflexes, the general anesthesia, the loss of consciousness, the cardio-in-

hibitory depression, the lowering of the blood pressure, — all are reversed and restored in a very short time and by a comparatively small quantity of a calcium salt. We do not know of any other instance in biology of such a striking, we may say miraculous, antagonistic effect.

We shall not attempt to offer a theory how this effect is accomplished. Any theory constructed from the present facts would be premature and would soon have to be reconstructed. The various hypotheses which one has, and has to have as a guide to direct the further search for facts, had better not see the light of day before their viability is well assured.

For the present we are burdened only with this one hypothesis, that magnesium favors inhibitory processes. All the facts which were brought out so far sustain it. Even the fact brought out by Loeb that *Polyorchis*, which is motionless in a pure sodium chloride solution, starts its rhythmical motions when a magnesium salt is added, means also inhibition and not excitation. The rhythmical swimming movements of this medusa are absent in the pure sodium chloride solution on account of the tonic state of the contractile tissues, which is manifested, according to Loeb, in a tonic contraction of the mouth and tentacles. These tonic contractions become relaxed, inhibited by the addition of magnesium.

Another exception to the inhibition hypothesis would seem to offer itself in a fact recorded in this paper, that the cardio-inhibitory effect of the vagus becomes greatly reduced under the influence of strong concentrations of magnesium salts. Here is an inhibitory function in the body which is not intensified by magnesium, but rather depressed. The obstacle, however, is in no way fatal, and some qualification of the hypothesis could be easily made which would then cover also this apparent exception. We prefer, however, to wait and collect more facts before we attempt to give a final form (or even then only provisionally final?) to this hypothesis.

We wish, however, to discuss briefly the nature of the action of calcium. In our present series of experiments calcium was anything but inhibitory; it promptly restored contractility and sensibility. Also, in Loeb's experiments on *Polyorchis*, calcium antagonizes magnesium.

Furthermore, as we have already mentioned, calcium restores the irritability of muscle and nerve lost through the effects of potassium, rubidium, etc., and restores indirect irritability of the



nerve lost through the influence of sodium, potassium, etc. In all these instances calcium antagonizes inhibitory effects and increases excitability. Is calcium an exciting agent? We know, on the other hand, that calcium inhibits the rhythmic contractions of the frog muscle brought on by the salts of sodium, rubidium, etc. (Loeb); it inhibits the rhythmic contraction of the centre of gonionemus caused by pure solutions of NaCl or NaBr (Loeb). It inhibits the tonic contractions of frog muscles brought on by the salts of potassium, ammonium, etc. (Zoethout). It apparently inhibits intestinal peristalsis (J. B. McCallum). Finally, the last days brought us the news of a remarkable inhibitory effect of calcium which might have important practical bearings. G. W. McCallum and Voegtlin<sup>15</sup> found that the tetany following the removal of the epithelial bodies (parathyroids) in dogs can be promptly inhibited by the intravenous injection of calcium chloride. Injections of potassium salts increase the tetany, but a little larger dose of calcium will again inhibit it.

We have, then, one series of facts in which calcium undoubtedly antagonizes abnormal inhibition, and another series of facts in which calcium antagonizes abnormal excitation. Is calcium an inhibitory or an exciting agent? Clearly it is both or neither. It seems to us that, for the present at least, we do best to stick to facts; and these are that calcium antagonizes the abnormal activity of its three steady companions in the body, Mg, Na, and K, be this abnormal activity exaggerated inhibition or exaggerated excitation.

"The idea to which we have given preference, namely, that the substitution of Na or K for Ca, or *vice versa*, in certain organic compounds, gives rise to a contraction, may possibly have to be modified in detail, and undoubtedly many new facts will be required and found before we are ready for a final theory; but I am inclined to believe that the main structure will remain such as intimated in my papers in 1899 and 1900; namely, that the normal qualities, especially the normal irritability, of animal tissues depend upon the presence in these tissues of Na-, K-, Ca-, and Mg-ions in the right proportion."<sup>16</sup>

Before concluding we may refer here to the interesting fact that O. Loew discovered some years ago, that the harmfulness of magnesium compounds to algæ and other plants can be neutralized by the

<sup>15</sup> McCALLUM and VOEGTLIN: Johns Hopkins Bulletin, 1908, March, p. 91.

<sup>16</sup> JACQUES LOEB: The dynamics of living matter, p. 94.

addition of calcium compounds. Furthermore, Benecke,<sup>17</sup> in studies upon Spirogyra, found that potassium is also a poison and that this poisonous effect can be neutralized by calcium. It seems, therefore, that *in animals as well as in plants calcium neutralizes the harmful effects of magnesium and perhaps also the poisonous effects of the other salts.*

#### CONCLUSIONS.

Intravenous infusion of various calcium salts is capable of completely reversing the pronounced inhibitory effects brought on by various magnesium salts. The respiratory paralysis, the lost lid reflex, the motor paralysis, the lost general reflexes, the general anesthesia, the loss of consciousness, the depression of the cardio-inhibitory action of the vagus, the lowering of the blood pressure, — all are reversed and completely restored in a very short time by the injection of a comparatively small quantity of a calcium salt. This statement does not hold good for conditions brought about by large doses of magnesium.

Calcium efficiently antagonizes the abnormal activity of its three inorganic associates in the animal body, Mg, K, and Na, be the activity an over-inhibition or an over-excitation.

The facts, as known at present, are still in harmony with the theory that magnesium favors essentially inhibitory processes in the animal body.

The antagonism of calcium to magnesium is a phenomenon common to animals and plants.

<sup>17</sup> W. BENECKE: Berichte der deutschen botanischen Gesellschaft, 1907, xxv, p. 322.

# A STUDY OF THE EQUILIBRIUM BETWEEN CARBONIC ACID, SODIUM BICARBONATE, MONO-SODIUM PHOSPHATE, AND DI-SODIUM PHOSPHATE AT BODY TEMPERATURE.

BY LAWRENCE J. HENDERSON AND O. F. BLACK.

[From the Laboratory of Biological Chemistry of the Harvard Medical School.]

IN a previous paper<sup>1</sup> we have reported results of the study of the equilibrium between carbonic acid, sodium bicarbonate, mono-sodium phosphate, and di-sodium phosphate at room temperature, and have shown that the nature of this equilibrium, necessarily one of those occurring in the typical cell, is such that approximate neutrality is safeguarded with great efficiency by the presence of such a group of substances.

The great importance of neutrality regulation in the organism, a regulation undoubtedly in large part dependent on this equilibrium,<sup>2</sup> together with the certainty that the reactions involved cannot be uninfluenced by the temperature, have now led us to a determination of the corresponding relationships at body temperature, in which care was taken to obtain results of accuracy more than sufficient for the purposes of physiological discussion. The outcome of these experiments is reported in the present paper. The results serve to characterize with great accuracy the equilibrium between free carbonic acid and the phosphates and carbonates of strong bases like sodium and potassium, at body temperature, under varying conditions of acidity and alkalinity; they therefore constitute a secure basis for the consideration of neutrality equilibrium in the true aqueous solutions of the body, whether intra-cellular or extra-cellular.

## THEORETICAL CONSIDERATIONS.<sup>3</sup>

In solutions containing mono-sodium phosphate and di-sodium phosphate, sodium bicarbonate, and carbonic acid the following ex-

<sup>1</sup> HENDERSON and BLACK: This journal, 1907, xviii, p. 250.

<sup>2</sup> See the following paper.

<sup>3</sup> For a detailed discussion see the following paper.

pression, deduced from the concentration law, indicates approximately the relations between the different substances. The adequacy of this definition is attested by the outcome of the present investigation.

$$(H) = k_1 \times \frac{NaH_2PO_4}{Na_2HPO_4} = k_2 \times \frac{H_2CO_3}{NaHCO_3};$$

whence

$$\frac{\frac{NaH_2PO_4}{Na_2HPO_4}}{\frac{H_2CO_3}{NaHCO_3}} = \frac{k_2}{k_1} = K.$$

In the equation  $k_1$ ,  $k_2$ , and  $K$  are all constants for any particular temperature; at  $18^\circ$  they are approximately equal to  $2.5 \times 10^{-7}$ ,  $3.8 \times 10^{-7}$ , and 1.5 respectively.

The data for the heats of ionization of the two acid substances together with the known ionization constant of water indicate that at  $38^\circ$

$$K > 1.5.$$

The data reported in this paper have yielded the following numbers for four different stages of the equilibrium.<sup>4</sup>

	I	II	III	IV
$R_1 = \frac{NaH_2PO_4}{Na_2HPO_4}$	1.29	1.09	0.46	0.195
$R_2 = \frac{H_2CO_3}{NaHCO_3}$	0.40	0.33	0.14	0.065

From the above table the following relationships are obtained:

$$K_{38^\circ} = \frac{R_1}{R_2} \quad \begin{array}{cccc} \text{I} & \text{II} & \text{III} & \text{IV} \\ 3.2 & 3.3 & 3.3 & 3.0 \end{array}$$

whence

$$K_{38^\circ} = 3.3.^5$$

<sup>4</sup> Each number is the average obtained from three or more determinations, very closely concordant. The numbers in column IV, for technical reasons, vary greatly as the result of very slight differences in the analytical determinations. Accordingly they carry less weight than the other values of  $R_1$  and  $R_2$ ; none the less is their accord with the theory a very satisfactory one.

<sup>5</sup> The value in column IV is disregarded in calculating this average; see the preceding note.

These results are indicated upon the accompanying diagram (Fig. 1). The close accord between theory and experiment is shown by the closeness of the experimentally determined points to the continuous straight line, which therefore accurately defines the equilibrium in moderately dilute solutions of phosphates and carbonates at 38°. For comparison the relationships at 18° are indicated by the broken line.

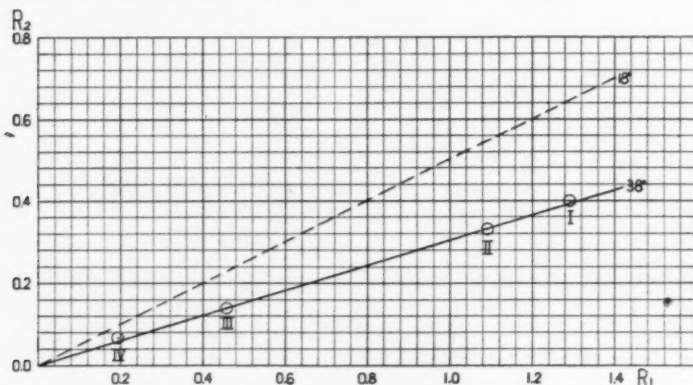


FIGURE 1.  $R_1 = \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4}$   $R_2 = \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$

This close approximation to constancy in numbers, which, being based upon small differences between experimentally determined quantities, are open to wide variations as the result of slight errors, is highly gratifying. Moreover it presents a guarantee of the accuracy of the experimental work, and of the justice of the approximation whereby the theoretical conclusions are developed.

#### EXPERIMENTAL PART.

For the investigation two solutions of carefully purified material were prepared, — one containing sodium bicarbonate, the other phosphoric acid. These solutions were carefully analyzed, the bicarbonate solution by titration against deci-normal hydrochloric acid, the phosphoric acid solution by titration against deci-normal sodium hydrate, and also gravimetrically by precipitation with ammonium molybdate.<sup>6</sup> The averages of closely concordant results were

<sup>6</sup> BAXTER: American chemical journal, 1902, xxviii, p. 298.



These solutions were used throughout the investigation.

In every experiment mixtures of the two solutions were saturated with a gas of known tension, consisting wholly or in part of carbon dioxide, in an apparatus of the following construction. A small separatory funnel was cut off just below the stopcock, and a glass bulb, itself fitted with another stopcock, was sealed on, thus forming a double separatory funnel (Fig. 2). The volume within the apparatus between the stopcocks was accurately determined by filling with distilled water at  $18^\circ$  and weighing the water. The average of closely agreeing determinations indicated that this volume was 30.62 c.c.

The experimental procedure was in every determination as follows: A mixture of sodium bicarbonate and phosphoric acid was made by drawing the desired quantities of the two solutions from burettes into a beaker. Here they were thoroughly mixed by stirring, and then by suction transferred into the above-described apparatus. Enough material was used to fill not only the bulb, but also the upper vessel to a depth of about 3 cm. The apparatus was next connected at its lower end with a generator of carbonic acid, or reservoir of gas, through a wash bottle. This bottle contained a solution of sodium chloride of approximately the same vapor tension as the mixture to be saturated. Both wash bottle and saturation apparatus were immersed in a thermostat which was maintained at  $37.5^\circ$ . A slow current of carbon dioxide or of a mixture of carbon dioxide and air was allowed to flow through wash bottle and saturation apparatus for one hour. The stopcocks were then closed, the bulb cooled to  $18^\circ$ , with an occasional opening of the upper stopcock to allow liquid to enter the space caused by contraction, and finally the upper bulb and the tube at the lower end of the

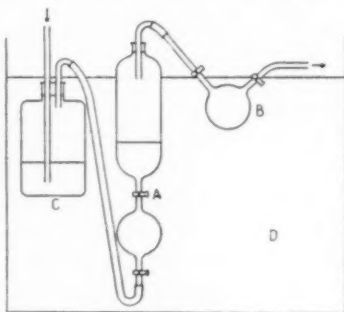


FIGURE II.—*A*, Saturation apparatus.  
*B*, Bulb for sampling gas mixture.  
*C*, Wash bottle. *D*, Water of thermostat.

apparatus were washed out with water. This gave a volume of solution equal to the capacity of the bulb at  $18^{\circ}$ , which contained all the carbonic acid dissolved by it during the experiment.

The next step was to determine the total carbonic acid in this measured volume of liquid. To this end the contents of the bulb were allowed to flow into a bottle filled with air free from carbon dioxide. Dilute sulphuric acid was next run into the bottle, and the gas evolved was carried through a drying-apparatus and thence through potash bulbs by a slow current of air in the customary way. The usual precautions to avoid entrance of carbonic acid from the air were observed; these precautions also prevented escape of carbonic acid from the solution. Finally, the gain in weight of the potash bulbs indicated the total carbonic acid of the solution. For purposes of comparison the carbonic acid absorbed by a solution of potassium chloride, approximately 0.05 N, under the conditions of the experiment, was also determined (Experiment A).

In the later experiments it was necessary to saturate the solution with a mixture of air and carbon dioxide in known proportions. Here difficulty was at first experienced, owing to the solubility of carbon dioxide in water. The use of mercury with the large volumes of gas required would have been inconvenient. Accordingly we prepared mixtures of carbon dioxide and air in large bottles by displacing known volumes of water with air and carbon dioxide in approximately the desired ratio. This mixture was then passed through the absorption apparatus as before, but at the exit was attached a bulb fitted with two stopcocks. This bulb was immersed in the water of the thermostat. The volume of the bulb being known, it was a simple matter, by analyzing its contents at the end of the experiment, to measure with considerable exactness the ratio of carbon dioxide to air in the gas used.

The data of the experiments are as follows:

*Experiment A.* —

Solution ; —	0.05 N KCl	
Average tension <sup>7</sup> CO <sub>2</sub> ; —	76 cm.	
CO <sub>2</sub> found ; —	0.0313	0.0335
	0.0321	0.0319
Average . . . . .	0.0322 gm.	

<sup>7</sup> The average tension of carbon dioxide is found by dividing the average barometric pressure by the average per cent by volume of carbon dioxide in the gas used for saturation.



*Experiment I. —*

Solution <sup>8</sup> ; — $\text{NaHCO}_3 : \text{H}_3\text{PO}_4 = 2 : 1$		
Average tension $\text{CO}_2$ ; — 76 cm.		
$\text{CO}_2$ found; —	0.1133	0.1137
	0.1135	0.1132
	0.1129	
Average . . . . .	0.1133 gm.	

*Experiment II. —*

Solution; — $\text{NaHCO}_3 : \text{H}_3\text{PO}_4 = 3 : 1$		
Average tension $\text{CO}_2$ ; — 76 cm.		
$\text{CO}_2$ found; —	0.1303	0.1313
	0.1307	
Average . . . . .	0.1308 gm.	

*Experiment III. —*

Solution; — $\text{NaHCO}_3 : \text{H}_3\text{PO}_4 = 2 : 1$		
Average tension $\text{CO}_2$ ; — 20.3 cm.		
$\text{CO}_2$ found; —	0.0876	0.0872
	0.0886	
Average . . . . .	0.0878 gm.	

*Experiment IV. —*

Solution; — $\text{NaHCO}_3 : \text{H}_3\text{PO}_4 = 2 : 1$		
Tension $\text{CO}_2$	$\text{CO}_2$ found	
12.2 cm.	0.0799 gm.	
13.0	0.0819	
11.2	0.0781	
10.1	0.0767	

From the data the following average molal concentrations are calculated:<sup>9</sup>

<sup>8</sup>  $\text{NaHCO}_3 = 0.1154 \text{ N.}$

$\text{H}_3\text{PO}_4 = 0.1055 \text{ N.}$

<sup>9</sup> The numbers are obtained as follows: The concentration of free carbonic acid,  $\text{H}_2\text{CO}_3$ , is calculated directly from the tension of carbonic acid, with the aid of the measurements of solubility of carbonic acid (Experiment A). The difference between total carbonic acid and free carbonic acid yields the value for concentration of sodium bicarbonate,  $\text{NaHCO}_3$ . The difference between the total amount of sodium bicarbonate introduced into the solution at the beginning of the experiment and the calculated concentration of bicarbonate gives the amount of sodium combined with phosphoric acid, which readily yields the concentrations of mono-sodium phosphate and di-sodium phosphate,  $\text{NaH}_2\text{PO}_4$ , and  $\text{Na}_2\text{HPO}_4$ , in the table.

	I	II	III	IV
$\text{NaH}_2\text{PO}_4$	0.0066	0.0046	0.0037	0.0019
$\text{Na}_2\text{HPO}_4$	0.0051	0.0042	0.0080	0.0098
$\text{H}_2\text{CO}_3$	0.0239	0.0240	0.0080	0.0037
$\text{NaHCO}_3$	0.0602	0.0732	0.0572	0.0564

In Experiments I, II, and III the conditions were satisfactory, and the calculated values of  $R_1$  and  $R_2$ , as indicated above, accurate. In Experiment IV the conditions were somewhat unsatisfactory, in that very small errors in the determinations of carbonic acid suffice greatly to affect the ratios between the several concentrations. An examination of the above table makes this clear, and justifies the neglect of Experiment IV in taking the average values.

It is a pleasure to record our indebtedness to the Proctor Fund for aid in this investigation.

#### SUMMARY.

A simple method is described for saturating solutions, at constant temperature, with carbon dioxide of known tension.

Experimental data are reported concerning the constitution, at body temperature, of aqueous solutions in which are present carbonic acid, sodium bicarbonate, mono-sodium phosphate, and disodium phosphate.

It is shown that in such solutions the equilibrium may be defined by the proportion:

$$\frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4} : \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = 3.3 : 1.0$$

This relationship corresponds to the requirements of the concentration law.

It is pointed out that these facts serve to define very accurately the most important equilibria of neutrality regulation in the aqueous solutions of the body.

## THE THEORY OF NEUTRALITY REGULATION IN THE ANIMAL ORGANISM.

BY LAWRENCE J. HENDERSON.

[From the Laboratory of Biological Chemistry of the Harvard Medical School.]

WITH the results reported in the preceding paper, the work which has been carried on in this laboratory concerning equilibria of neutrality<sup>1</sup> during the last three years has progressed far enough to make possible a general discussion of the equilibria between phosphates and carbonates in the body of the warm-blooded animal. Such is the purpose of the present paper.

### THE SIMPLE EQUILIBRIUM.

In the ionization reaction of a weak acid,  $HA = H^+ + A^-$ , the conditions of equilibrium are defined by the equation of the concentration law,

$$k \cdot (HA) = (H^+) \cdot (A^-),$$

in which the enclosed quantities stand for concentrations, and  $k$  is the ionization constant of the acid. This equation may more conveniently be written as follows:

$$(H^+) = k \times \frac{(HA)}{(A^-)}.$$

If an aqueous solution of such an acid and its sodium salt be prepared, it is evident that here the concentration of the undissociated molecules of the acid ( $HA$ ) will be almost precisely equal to the total amount of acid present, for such an acid under these conditions will be hardly at all ionized. On the other hand, the concentration

<sup>1</sup> HENDERSON: This journal, 1906, xv, p. 257; 1908, xxi, p. 169; 1908, xxi, p. 173. HENDERSON and BLACK: This journal, 1907, xviii, p. 250; 1908, xxi, p. 420. FITZ, ALSBERG, and HENDERSON: This journal, 1907, xviii, p. 250. HENDERSON and WEBSTER: The journal of medical research, 1907, xvi, p. 1.

of the ion, ( $\bar{A}$ ), will be equal to the concentration of the salt multiplied by its degree of ionization. This last quantity varies with the nature of the acid and with the concentration; it is also affected by the presence of other salts in solution. For present purposes, however, it may be estimated as 0.8 without seriously impairing the conclusions. This estimate can hardly be more than 10 per cent to 15 per cent in error in any of the solutions here discussed.

We may then write, as an approximation sufficiently accurate for our present purposes, the equation

$$(\bar{H}) = \frac{k}{0.8} \times \frac{HA}{NaA};$$

that is to say, in the solution of a weak acid and its sodium salt, the concentration of hydrogen ions is equal to the ionization constant of the acid divided by 0.8, approximately the degree of ionization of the salt, and multiplied by the ratio between the total amounts of acid and salt present in the solution.

This equation may be used in analyzing the nature of the equilibrium between carbonic acid and sodium bicarbonate, and also for the equilibrium between mono- and di-sodium phosphates.

The ionization constant of carbonic acid, as determined by Walker<sup>2</sup> at 18°, is  $3.04 \times 10^{-7}$ . For the ion  $\bar{H}_2\text{PO}_4$  at 18° the ionization constant has been determined by Abbott<sup>3</sup> to be  $2.01 \times 10^{-7}$ . Accordingly we may write the equations

$$(\bar{H}) = \frac{3.0 \times 10^{-7}}{0.8} \times \frac{H_2CO_3}{NaHCO_3}$$

$$(\bar{H}) = \frac{2.0 \times 10^{-7}}{0.8} \times \frac{NaH_2PO_4}{Na_2HPO_4},$$

whence

$$(\bar{H}) = 3.8 \times 10^{-7} \times \frac{H_2CO_3}{NaHCO_3}$$

$$(\bar{H}) = 2.5 \times 10^{-7} \times \frac{NaH_2PO_4}{Na_2HPO_4}.$$

As has been shown elsewhere independently by Washburn<sup>4</sup> and myself,<sup>5</sup> that combination of acid and salt in which the ionization constant of the acid, divided by the degree of ionization of the salt,

<sup>2</sup> See MCCOY: American chemical journal, xxix, p. 437.

<sup>3</sup> Verbal communication of Professor A. A. NOYES.

<sup>4</sup> WASHBURN: Journal of the American Chemical Society, 1908, xxx, p. 31.

<sup>5</sup> HENDERSON: This journal, 1908, xxi, p. 173.

is equal to the square root of the water constant, or  $0.8 \times 10^{-7}$  at  $18^\circ$ , is most efficient to preserve neutrality in simple aqueous solution. Therefore in simple solution the two pairs of substances above considered approach closely to the highest efficiency in preserving neutrality; in the body, as we shall see, this efficiency is greatly increased.

With the aid of the principle of isohydric solutions, which states that when two solutions possess a common ion in the same concentration, mixing the solutions cannot change the concentration of the common ion, it is possible to calculate the equilibrium at  $18^\circ$  between the four substances carbonic acid, sodium bicarbonate, mono-sodium phosphate, and di-sodium phosphate. Thus, at a hydrogen ionization of  $1 \times 10^{-7}$ , we may write the equations

$$\frac{H_2CO_3}{NaHCO_3} \times 3.8 \times 10^{-7} = \frac{NaH_2PO_4}{Na_2HPO_4} \times 2.5 \times 10^{-7} = 1.0 \times 10^{-7};$$

whence  $\frac{H_2CO_3}{NaHCO_3} = \frac{1}{3.8}$  and  $\frac{NaH_2PO_4}{Na_2HPO_4} = \frac{1}{2.5}.$

That is to say, a solution possessing the hydrogen ion concentration  $1 \times 10^{-7}$ , which contains the above four substances, must contain 3.8 times as much sodium bicarbonate as carbonic acid, and 2.5 times as much di-sodium phosphate as mono-sodium phosphate. Obviously the concentrations of these two pairs of substances may vary greatly, provided the two ratios do not vary, without materially affecting the hydrogen ionization. The latter quantity will be affected in such a case only by variation in the degree of ionization of the salt. By similar calculations the equilibria for other degrees of hydrogen ionization may be calculated. The results of these calculations are confirmed with a sufficient degree of accuracy by the analyses of such solutions, as carried out by Black and myself.<sup>6</sup> These results, theoretical and experimental, are shown upon the accompanying diagram (Fig. 1). The diagram represents the conditions of equilibrium in a system of constant free carbonic acid concentration, this concentration being proportional to one-half large division of the scale, in which the amount of phosphoric acid in the two forms,  $NaH_2PO_4$  and  $Na_2HPO_4$ , is constant. Thus it corresponds roughly to the conditions in the body; for here, except by diffusion of monophosphate, the total amount of phosphoric

<sup>6</sup> This journal, 1907, xviii, p. 250.

acid will hardly vary, and there is an almost unlimited supply of carbonic acid at a tension which is known to vary not very widely about an average value. Concentrations of sodium bicarbonate, expressed in terms of the concentration of free carbonic

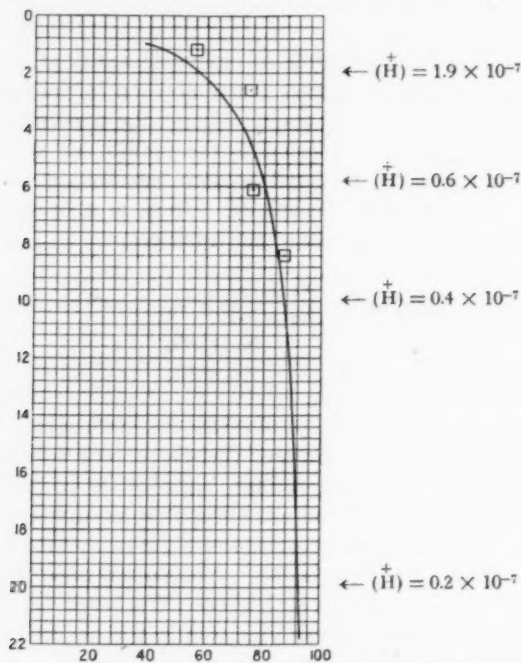


FIGURE 1.—The equilibrium at  $18^\circ (\text{H}^+) \times (\text{OH}^-) = 0.64 \times 10^{-14}$ . The curve indicates the calculated relationships. Experimentally determined points are indicated thus  $\square$ . The ordinates represent  $\frac{\text{NaHCO}_3}{\text{H}_2\text{CO}_3}$ ; the abscissæ show the per cent of phosphate as  $\text{Na}_2\text{HPO}_4$ .

acid, are plotted from above downward, concentrations of di-sodium phosphate, expressed in per cent of total phosphate, from left to right, and concentrations of mono-sodium phosphate, which varies inversely with di-sodium phosphate concentrations, are therefore indicated from right to left. Variations in these quantities are dependent upon the amount of sodium at the disposal of phosphoric and carbonic acids, and this amount may be varied by the addition of any alkaline substance, thus increasing it, or by the

addition of any acid substance, thus diminishing it, and thereby producing changes in equilibrium which are defined by the curve, — movement from above downward on the curve corresponding to increasing alkalinity, while movement in the opposite direction corresponds to increasing acidity. The hydrogen ion concentration at several points on the curve is also indicated. From a consideration of the diagram it is clear that with constant carbonic acid tension, corresponding to constant concentration of carbonic acid, according to the law of Henry, and thus roughly simulating conditions in the organism, there must be, at a hydrogen ionization  $0.3 \times 10^{-7}$ , which is nearly the hydrogen ionization of blood at room temperature, such a condition that very slight variations in hydrogen ionization must cause very great variations in sodium bicarbonate content, influencing at this point the ratio between mono- and di-sodium phosphate to a less degree. The result of that is evident; if to such a system, natural or artificial, either acid or alkali be added, an amount nearly commensurate with the sodium bicarbonate concentration must be employed materially to influence the hydrogen ionization. For instance, if a solution decimolal (that is to say, containing one tenth of a gram-molecule per litre) in respect to total carbonic acid and to total phosphoric acid possesses a hydrogen ionization of  $0.5 \times 10^{-7}$ , approximately the conditions hold that

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} \times 3.8 \times 10^{-7} = \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4} \times 2.5 \times 10^{-7} = 0.5 \times 10^{-7};$$

whence 
$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{1}{7.6} \quad \text{and} \quad \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4} = \frac{1}{5}.$$

Thus the concentration of  $\text{H}_2\text{CO}_3$  must be about 0.012 molal, of  $\text{NaHCO}_3$  about 0.088 molal, of  $\text{NaH}_2\text{PO}_4$  about 0.017 molal, and of  $\text{Na}_2\text{HPO}_4$  about 0.083 molal.

Let us investigate the conditions which it is necessary to fulfil in order to produce, from the above system, a hydrogen ionization of  $1 \times 10^{-7}$  N and a hydrogen ionization of  $0.2 \times 10^{-7}$  N, keeping the concentration of free carbonic acid constant.<sup>7</sup>

In the first instance we have the equations

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{1}{3.8} \qquad \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4} = \frac{1}{2.5}.$$

<sup>7</sup> These conditions are probably not far from the limits of variation in the body as a whole.



Here the concentration of sodium bicarbonate will be about 0.046 molal, and that of di-sodium phosphate about 0.072 molal. There must then have been added

$$(0.082 - 0.046) + (0.083 - 0.072) = 0.047$$

gram-molecule of acid per litre, or about one half as much acid as there was present sodium bicarbonate.

In the second instance the relationship is thus expressed:

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{1}{19} \qquad \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4} = \frac{1}{12.5}$$

Here the concentration of sodium bicarbonate becomes about 0.228 molal, and that of di-sodium phosphate about 0.093 molal. Thus there must have been added  $(0.228 - 0.088) + (0.093 - 0.083) = 0.150$  gram-molecule of alkali per litre, or about twice as much alkali as there were present sodium bicarbonate molecules. Thus is explained, with considerable precision, the enormous capacity of blood and protoplasm to preserve their neutrality.

By reference to the above curve it is evident that in blood and protoplasm the concentration of bicarbonates must be from ten to twenty times as great as the concentration of carbonic acid, for these are the relationships at hydrogen ion concentrations corresponding to those found in normal blood. Thus, inasmuch as the free carbonic acid of blood, as calculated from tension and absorption coefficient,<sup>8</sup> is known to be about one fifteenth of its total carbonic acid, on the average, it is evident that we have gained a strict proof that carbonic acid is present in blood plasma very largely, if not almost exclusively, as carbonic acid, sodium bicarbonate, and particularly the ion  $\text{HCO}_3^-$ . These relationships also serve as a further support of the theoretical considerations.

In the system defined by the above curve exact neutrality is only attained when the concentration of bicarbonate has fallen nearly to the concentration of carbonic acid. This offers a striking explanation of some of the phenomena of acidosis. Certainly it is not a strain upon one's conceptions of the nature of the metabolic processes to assert that a very great diminution in the carbonic acid content of blood cannot occur without gravely compromising these processes, in that removal of carbonic acid from the body must thus be

<sup>8</sup> BOHR, in NAGEL, *Handbuch der Physiologie*, i, erste Hälfte, p. 107.

greatly restricted, and it is fair to assume that too great diminution in the carbonic acid content of the blood must cause death from retention of carbonic acid. If, then, even exact neutrality of blood and protoplasm be impossible without the removal from blood of much more than half of its carbonic acid, it is reasonable to say that acidity, even in the slightest significant degree, of blood and protoplasm generally, is impossible during life. Local happenings are not here considered. On the other hand, with a constant source of carbonic acid which must inevitably convert into bicarbonates any bases which may be formed or introduced into blood and protoplasm, it is clear that increase in alkalinity must be slow, and material increase impossible. Thus to halve the hydrogen ion concentration, that is to say, to double the alkalinity, carbonic acid content remaining constant,<sup>9</sup> the amount of bicarbonate present, already very considerable as the curve indicates, must be doubled; further increase in bicarbonate concentration could hardly go on, if for no other reason, because it would soon result in an abnormally high osmotic pressure, and diffusion, aided by renal activity, would ultimately check the very slowly growing alkalinity. Indeed, a solution made by passing carbonic acid, of tension approximately equal to that in the blood, through a solution of sodium hydrate, such that the resulting system should possess a molecular concentration equal to that of blood, would possess the very slight alkalinity  $16 \times 10^{-6}$  hydroxyl ion concentration at room temperature.

The analysis of this simple equilibrium, then, clearly shows that, under conditions which we believe to be essential to the life of higher organisms, sensible degrees of acidity and of alkalinity, as measured at room temperature, are absolutely impossible during life in the body as a whole. It cannot be too strongly emphasized that the inevitable slight inaccuracies of calculation and determination of the equilibria here involved are as nothing compared with the difference between the enormous variations in amounts of base and acid, and the almost infinitesimal variations in hydrogen and hydroxyl ionization in such systems as those here considered. A further analysis of conditions may be cited to justify this conclusion. In the

<sup>9</sup> Throughout this paper it is assumed, for the sake of simplicity, that the concentration of dissolved carbonic acid in the body fluids remains constant. Actually that concentration does vary considerably, locally perhaps greatly, but always little compared with concentration of sodium bicarbonate. For that reason the assumption is allowable.

system above considered at a hydrogen ionization of  $2 \times 10^{-7}$  there are present approximately 0.16 mole of acid per litre and approximately 0.19 mole of base, and at a hydrogen ionization of  $0.5 \times 10^{-7}$  approximately 0.20 mole of acid and 0.27 of base. Thus in such a system, supplied with carbonic acid at constant tension, in order to change the acidity from that of a solution of hydrochloric acid of concentration 0.0000002 N to the alkalinity of a solution of sodium hydrate 0.0000002 N, it is necessary to add a decinormal solution of sodium hydrate nearly equal to the volume of the system. Clearly errors of even 100 per cent in the numbers in no way influence the justice of the conclusions.

If, in developing these considerations, weight has been laid chiefly upon the carbonates, that is not to say that they play a greater rôle than the phosphates in the matter. Salts of phosphoric acid are commonly the chief saline constituents of the cell, and the analysis of the transverse component of the above curve indicates that within the ranges of hydrogen ionization encountered in the body, particularly as neutrality is more closely approached, they also operate largely in neutralizing acid or alkali. Thus, a system possessing a hydrogen ionization of  $0.5 \times 10^{-7}$ , and decinormal with respect to total phosphoric acid and to total carbonic acid, must contain about 85.5 per cent of its phosphoric acid as di-phosphate, and about 89.5 per cent of its carbonic acid as bicarbonate. In this system, keeping the free carbonic acid constant, the addition of acid will produce a solution of hydrogen ionization  $1 \times 10^{-7}$  per litre of solution when di-sodium phosphate has neutralized 0.011 mole of a mono-basic acid, and sodium bicarbonate 0.047 mole. In passing from this condition to a hydrogen ionization of  $2 \times 10^{-7}$ , di-sodium phosphate must neutralize per litre 0.015 mole of acid, and sodium bicarbonate 0.023 mole of acid, while in passing from this ionization to  $4 \times 10^{-7}$  hydrogen ion concentration, the phosphate must neutralize 0.017 mole of acid and the carbonate only 0.009 mole. Moreover, in protoplasm the concentration of phosphates, on the average, is probably at least twice as great as that of carbonates, and thereby their effectiveness is proportionally increased. In addition to these considerations, the great diffusibility of acid phosphates, as shown by Maly,<sup>10</sup> in conjunction with the fact that acid has constantly to be removed from the body, seems to be a matter of great importance in neutrality regulation. In this connection it has been shown by

<sup>10</sup> MALY: *Berichte der deutschen chemischen Gesellschaft*, 1876, ix, p. 164.

Fitz, Alsberg, and Henderson,<sup>11</sup> that in rabbits the feeding of hydrochloric acid daily during a long period produces first a marked increase in the excretion of phosphoric acid by the kidney; this is then followed by a decrease in the excretion and by death. These experiments support the idea that acid introduced into the body, after being neutralized in part by di-phosphates, depends upon mono-phosphates as a principal vehicle for its removal. Such considerations as those above regarding heterogeneous equilibria are discussed below in full.

The relationships above developed are the underlying ones in the neutrality regulation within the aqueous solutions of the body, for no other substances which possess nearly the same power of preserving neutrality are there present in important amounts. There are, however, in the body certain factors which modify these fundamental considerations.

#### THE INFLUENCE OF TEMPERATURE ON THE EQUILIBRIUM.

In one important respect the physical conditions in the body differ from those above considered; the temperature at which the equilibrium is adjusted is not 18°, but 38°.

Theoretically it is possible to calculate the change in the equilibrium produced by changing temperature according to the well-known equation,

$$\log k_{T_2} - \log k_{T_1} = -\frac{q \times (T_2 - T_1)}{4.584 T_1 T_2},$$

where  $k$  stands for the ionization constant,  $T_1$  for the lower temperature,  $T_2$  for the higher temperature, and  $q$  for the heat of ionization in gram calories, 4.584 being the gas constant  $R$ , expressed in gram calories, divided by the factor to convert natural logarithms into common logarithms.

In this case

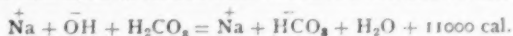
$$T_2 = 38^\circ + 273^\circ = 311^\circ$$

$$T_1 = 18^\circ + 273^\circ = 291^\circ$$

For carbonic acid  $k_{18^\circ}$  is  $3.04 \times 10^{-7}$  and  $q$ , according to Thomsen, -2750 cal., and for the ion  $\text{H}_2\text{PO}_4$   $k_{18^\circ}$  is  $2.01 \times 10^{-7}$  and  $q$  is -1500.<sup>12</sup>

<sup>11</sup> FITZ, ALSBERG, and HENDERSON: This journal, 1907, xviii, p. 113.

<sup>12</sup>  $q$  may be calculated as follows from Thomsen's well-known data:



Thus we obtain the equations for carbonic acid,

$$\log k_{38^\circ} = -7 + 0.491 + \frac{2750 \times 20}{4.584 \times 291 \times 311},$$

and for the ion  $\text{H}_2\text{PO}_4^-$ ,

$$\log k_{38^\circ} = -7 + 0.301 + \frac{1500 \times 20}{4.584 \times 291 \times 311},$$

or for carbonic acid,

$$k_{38^\circ} = 4.2 \times 10^{-7},$$

and for the ion  $\text{H}_2\text{PO}_4^-$ ,

$$k_{38^\circ} = 2.4 \times 10^{-7}.$$

That is to say, both acids have increased in strength, carbonic acid more than mono-sodium phosphate, as a result of rise in temperature.

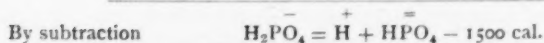
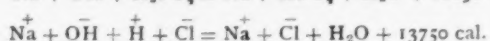
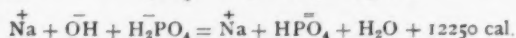
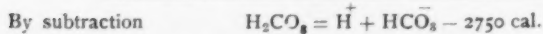
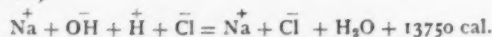
Unfortunately these calculations can hardly be of much value, save to indicate the general nature of the changes in ionization constants as the temperature rises from  $18^\circ$  to  $38^\circ$ , for they are based upon differences between heats of reaction whose magnitude is not a little in doubt. Accordingly the magnitudes of the differences are very greatly in doubt.

It is possible, however, to determine at least the ratio between the two constants at  $38^\circ$  without difficulty, with the aid of the data presented in the preceding paper.<sup>12</sup> The following numbers are taken from that source:

	I	II	III
$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$	0.40	0.33	0.14
$\frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4}$	1.29	1.09	0.46

$$\text{But } (\text{H}) = \frac{k_{\text{H}_2\text{CO}_3}}{0.8} \times \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{k_{\text{H}_2\text{PO}_4}}{0.8} \times \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4};$$

<sup>12</sup> (continued).



<sup>13</sup> HENDERSON and BLACK: This journal, 1908, xxi, p. 420.

whence

$$\frac{k_{\text{H}_2\text{CO}_3}}{k_{\text{H}_2\text{PO}_4}} = \frac{\frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4}}{\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}}$$

From this relationship and the numbers in the above table the following values for the ratio, *R*, between the ionization constants at 38° of carbonic acid and of the ion H<sub>2</sub>PO<sub>4</sub>, are calculated.

	<i>R</i>
I	3.2
II	3.3
III	3.3
Average	3.3.

This result is probably very much more accurate than the values for the constants which were obtained above by calculation, with the aid of the thermo-chemical data concerning phosphoric acid and carbonic acid. Using the average number 3.3 for the ratio, the accompanying diagram is constructed, after the fashion of Fig. 1; on this diagram the equilibrium in solution at 38° between the four substances under consideration is represented (Fig. 2).

The hydrogen ionization in this system has not been determined; it must, however, be very near the hydrogen ionization in the system at 18°, and its values may be estimated, as a first approximation, according to the calculation with the aid of the heat of ionization of carbonic acid which is above carried out. The values thus calculated for hydrogen ion concentrations are indicated upon the diagram. The curve shows with great precision the relationships between carbonates and phosphates which obtain in true aqueous solutions, whether intra-cellular or extra-cellular, in the body. Thus it constitutes a precise definition of one of the fundamental equilibria within the typical cell. An analysis shows that for any given ratio of carbonic acid to sodium bicarbonate the per cent of phosphoric acid as di-sodium phosphate is somewhat diminished, on the average about 12 per cent, as compared with the equilibrium at 18°. Coincidentally the hydrogen ion concentration is slightly increased; otherwise the quantitative relationships are almost identical with those discussed for the equilibrium at 18°, and accordingly further consideration of the case is superfluous, save in one important respect, the concentration of hydroxyl ions.

It has long been known that the heat of ionization of water is very great, and accordingly that the ionization constant of water  $C_{H_2O} = (H^+) \times (OH^-)$  varies greatly with the temperature. This variation may be calculated from conductivity measurements of

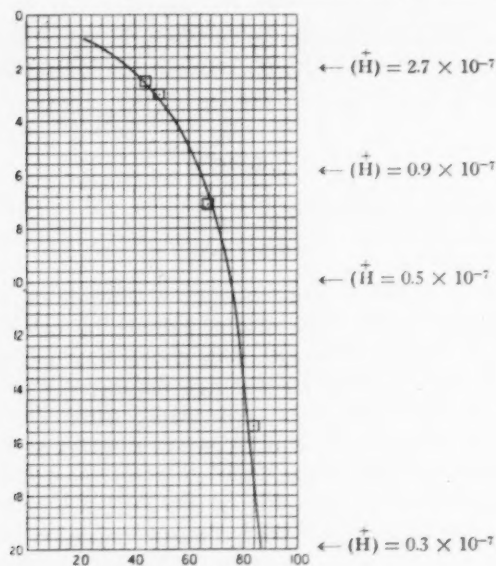


FIGURE 2.—The equilibrium at  $38^\circ$   $(H^+) \times (OH^-) = 2.9 \times 10^{-14}$ . The curve indicates the calculated relationships (from the average of the experimental values). Points experimentally determined are indicated thus:  $\square$ . The abscissæ represent  $\frac{NaHCO_3}{H_2CO_3}$ ; the ordinates show the per cent of phosphate as  $Na_2HPO_4$ .

Kohlrausch and Heydweiller,<sup>14</sup> and from their determinations the following numbers, important in this discussion, are taken directly or by interpolation.<sup>15</sup>

T	$C_{H_2O}$
$18^\circ$	$0.64 \times 10^{-14}$
$38^\circ$	$2.90 \times 10^{-14}$
$42^\circ$	$3.76 \times 10^{-14}$

<sup>14</sup> KOHLRAUSCH and HEYDWEILLER: WIEDEMANN'S Annalen, 1894, liii, p. 209.

<sup>15</sup> If the value of the constant at  $18^\circ$  in the table is assumed to be accurate, it is possible to calculate the value of the constant at  $38^\circ$ , as above in the case of carbonic acid and of the ion  $H_2PO_4^-$ . The result of this calculation is  $3.3 \times 10^{-14}$ .



A solution of carbonic acid and sodium bicarbonate which at 18° possesses a hydrogen ionization of  $0.30 \times 10^{-7}$  would have, according to Thomsen's data for the heat of ionization of carbonic acid, at 38° a hydrogen ionization of  $0.40 \times 10^{-7}$ , and at 42° a hydrogen ionization of  $0.42 \times 10^{-7}$ . The hydroxyl ionization may be calculated as follows for such a solution:

$$(\text{OH}) = \frac{C_{\text{H}_2\text{O}}}{(\text{H})}$$

$$(\text{OH})_{18^\circ} = \frac{0.64 \times 10^{-14}}{0.30 \times 10^{-7}} = 2.1 \times 10^{-7}$$

$$(\text{OH})_{38^\circ} = \frac{2.90 \times 10^{-14}}{0.40 \times 10^{-7}} = 7.2 \times 10^{-7}$$

$$(\text{OH})_{42^\circ} = \frac{3.76 \times 10^{-14}}{0.42 \times 10^{-7}} = 9.0 \times 10^{-7}$$

This calculation indicates that at 38° any moderately dilute solution of carbonic acid and sodium bicarbonate has an alkalinity about 3.4 times as great as at 18°, and at 42° a hydroxyl ionization about 4.3 as great as at 18°. When the temperature rises from 38° to 42°, its increase in alkalinity is about one fourth. These relatively large and important changes in hydroxyl ion concentration are due to the fact that the ionization constant of water increases with rising temperature much more rapidly than does the ionization constant of carbonic acid.

Every solution containing sodium bicarbonate and carbonic acid must, according to these conclusions, become more alkaline as the temperature rises, because its hydrogen ionization is kept nearly constant, unless by reaction with other substances present in the solution the sodium bicarbonate content diminishes. Such a solution, possessing at 18° a hydrogen ionization  $0.3 \times 10^{-7}$ , contains thirteen times as much sodium bicarbonate as carbonic acid. To preserve its alkalinity constant, it must at 38° contain only about 3.5 times as much sodium bicarbonate as free carbonic acid. Accordingly, if the carbonic acid concentration does not vary, nearly three fourths of the sodium bicarbonate must have reacted with an acid to form the sodium salt of the acid, and carbonic acid, which has escaped.

It is safe to conclude from this consideration that solutions containing considerable amounts of carbonic acid and sodium bicarbonate will nearly always grow relatively much more alkaline as

the temperature rises, though of course their absolute alkalinity remains very low. Blood is such a solution, and the proof of such a relationship in blood is a matter of no small importance both for physiology and for pathology. To this end my collaborator, Mr. O. F. Black, and I have made preliminary measurements of the hydrogen ionization of blood at different temperatures, using

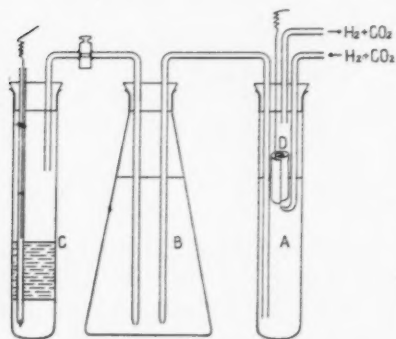


FIGURE 3. — Diagram of concentration cell. A, Blood. B, Salt solution. C, Calomel electrode. D, Hydrogen electrode.

a concentration cell of the following construction. Rabbit's blood was brought in contact with a platinized platinum hydrogen electrode, and through the blood was passed a gaseous mixture consisting of 95 parts of hydrogen and 5 parts of carbon dioxide, measured by volume. The blood was connected with a normal sodium chloride solution, and this in turn with a calomel electrode. The arrangement is shown in the accompanying figure (Fig. 3).

Measurements were made in the usual way against a cadmium element of electromotive force  $\pi = 1.019$  volts. The results, whose absolute magnitudes are obviously somewhat in error, perhaps owing to the uncertainty of the hydrogen electrode, are probably quite reliable relatively, and we are here concerned only with relative values. They indicate that the hydrogen ionization of blood increases very little with the temperature, and nearly in the expected degree.

$$\begin{array}{ll}
 \text{I} & \left\{ \begin{array}{l} 22^\circ \text{ } ^+\text{(H)} = 2.6 \times 10^{-9} \\ 39^\circ \text{ } ^+\text{(H)} = 3.1 \times 10^{-9} \end{array} \right. \\
 \text{II} & \left\{ \begin{array}{l} 20^\circ \text{ } ^+\text{(H)} = 9.6 \times 10^{-9} \\ 51^\circ \text{ } ^+\text{(H)} = 12.0 \times 10^{-9} \end{array} \right. \\
 \text{III} & \left\{ \begin{array}{l} 19^\circ \text{ } ^+\text{(H)} = 4.4 \times 10^{-9} \\ 39^\circ \text{ } ^+\text{(H)} = 5.8 \times 10^{-9} \end{array} \right. \\
 \text{IV} & \left\{ \begin{array}{l} 24^\circ \text{ } ^+\text{(H)} = 3.3 \times 10^{-9} \\ 39^\circ \text{ } ^+\text{(H)} = 4.0 \times 10^{-9} \end{array} \right.
 \end{array}$$

At the same time that the above results were obtained a solution made by mixing 19 parts of normal sodium chloride solution and 1 part of normal sodium bicarbonate solution was investigated; for this solution the value was found at 39°,

$$(H) = 6.8 \times 10^{-9}.$$

This result bears out the evident fact that the above numbers are too low;<sup>16</sup> it presents, however, a strong support to the belief that their relative magnitudes have been measured with sufficient accuracy for the present purpose.

The average rise in hydrogen ion concentration per degree rise in temperature in these experiments is 1.2 per cent, corresponding to a rise of 25 per cent for the temperature interval between 18° and 38°, or very nearly what is to be expected for a pure solution of sodium bicarbonate and carbonic acid. It is safe to conclude, therefore, that the alkalinity of blood, and probably also of protoplasm, increases materially with rising temperature, indeed almost as much as the water constant itself.<sup>17</sup>

Unless the relative magnitudes of the above results are very greatly in error, a condition which could only have been due to a very great diminution in the sodium bicarbonate content of the blood as the temperature rises, blood in the body is about three times as alkaline, that is to say, three times as rich in hydroxyl ions, as it has been generally believed to be.<sup>18</sup> Moreover it is very improbable that the concentration of sodium bicarbonate in blood diminishes to an important degree on raising the temperature of blood from 18° to 38°, provided the tension of carbonic acid is kept constant, for the only substances present in blood which can combine with sodium in considerable amounts are the proteins.

It is known that the concentration of the sodium protein compounds of blood serum at 18° is a small fraction of the sodium bi-

<sup>16</sup> This investigation will be continued, and it is hoped to obtain accurate measurements of the hydrogen ion concentration at different temperatures. It seems, however, worth while to present these purely preliminary results here for the sake of completeness in the discussion.

<sup>17</sup> Because the water constant which is equal to hydrogen ion concentration multiplied by hydroxyl ion concentration increases several hundred per cent, while the hydrogen ion concentration remains nearly constant.

<sup>18</sup> HÖBER: *Physikalische Chemie der Zelle und der Gewebe*, zweite Auflage, p. 152.

carbonate concentration, perhaps somewhat less than one tenth.<sup>19</sup> A diminution of one half in the sodium bicarbonate concentration would therefore increase the sodium protein compounds about five-fold, and it is highly improbable that such a change could occur without a marked increase in the very low alkalinity of the solution. On the other hand, it is not improbable that with the rising temperature enough sodium may be transferred from carbonic acid to proteins,<sup>20</sup> materially to increase the sodium protein concentration. This, however, would probably be offset, carbonic acid tension

being constant, in its effect on the ratio  $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$  by the diminution of concentration of dissolved carbonic acid, due to the diminution of the absorption coefficient of carbonic acid as the temperature rises. For plasma at 15° this coefficient is 0.994, and at 38° 0.541.<sup>21</sup>

Finally, it is clear that if the increase of alkalinity of blood as the temperature rises is of the order of magnitude indicated by both theory and experiment, which, as we have seen, are in very good accord, the increase of alkalinity of blood when the body temperature rises to that of high fever must be not inconsiderable (about 25 per cent), and probably not insignificant in its effects.<sup>2</sup>

#### THE HETEROGENEOUS EQUILIBRIUM.

In a previous paper<sup>22</sup> it has been shown, both theoretically and experimentally, that acids whose ionization constants are nearly equal to the concentration of hydrogen ions in a neutral solution possess, with the help of their salts, a great capacity of preserving neutrality. Other acids are, in like concentration, of relatively very little effect in this matter. Other things being equal, as it appears from the previous investigation, the greatest possible efficiency in preserving neutrality within small variations on both sides of the exact neutral point is possessed by that acid whose ionization constant, divided by the degree of ionization of its salt, is precisely equal to the hydrogen ion concentration in pure water.<sup>23</sup> But in the

<sup>19</sup> HENDERSON: This journal, 1908, xxi, p. 169.

<sup>20</sup> *Ibid.*

<sup>21</sup> BOHR, in NAGEL'S Handbuch der Physiologie, i, erste Hälfte, p. 63.

<sup>22</sup> HENDERSON: This journal, 1908, xxi, p. 173.

<sup>23</sup> That is to say, if  $k$  be the ionization constant of the acid and  $\gamma$  the degree of ionization of the salt, the best condition is thus defined:  $\frac{k}{\gamma} = \sqrt{C_{\text{H}_2\text{O}}}$ .

body the requirements are not precisely that variation in hydroxyl and hydrogen ion concentrations on both sides of the neutral point shall be held equally well in check. Rather is it necessary to preserve the hydrogen ion concentration at about  $0.3 \times 10^{-7}$  or  $0.4 \times 10^{-7}$ .

In a simple system such regulation could most efficiently be accomplished by an acid of ionization constant about  $0.2 \times 10^{-7}$ . The conditions in the body are, however, greatly modified from the simplest case of an isolated solution by the fact that carbonic acid is to be had in considerable amount at a nearly constant concentration, and by other similar factors already mentioned in the first part of this paper. As we have seen above, the ratio of acid to salt in blood and protoplasm is expressed approximately by the following proportion:

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{1}{14}.$$

But owing to the fact that the carbonic acid tension does not vary to a considerable extent under normal circumstances, material changes in the ratio, which is proportional to the hydrogen ionization, can be produced only by greatly varying the amount of sodium bicarbonate. Thus it appears that sodium bicarbonate and carbonic acid, under these circumstances, are much more effective in the regulation of the reaction than an acid of ionization constant  $0.2 \times 10^{-7}$  and its salt could be. For instance, the amount of acid which can be neutralized by bicarbonate in a system made up of carbonic acid in fixed concentration 0.0017 N and sodium bicarbonate, in passing from a hydrogen ionization  $0.2 \times 10^{-7}$  to a hydrogen ionization  $0.4 \times 10^{-7}$ , is about 0.018 mole per litre. The acid neutralized in passing from the former to the latter hydrogen ion concentration in a system made up of an acid of ionization constant  $0.2 \times 10^{-7}$  and its sodium salt, the concentration of the acid, free and combined, being equal to that of free and combined carbonic acid in the above system at that point where the hydrogen ion concentration is  $0.3 \times 10^{-7}$ , is only about 0.005 mole per litre.

Thus it appears that, through the nature of carbonic acid, and the fact that there is in the body a nearly constant supply of it, this substance possesses a quite wonderful capacity, more than three times as great as any other substance not thus regulated by a constant supply could have, of preserving the reaction in the neighborhood of a hydrogen ion concentration  $0.3 \times 10^{-7}$ .

This efficiency evidently depends in great part on the regulation of respiration, whereby the concentration of carbonic acid throughout the body is kept down, and upon those unknown mechanisms which check carbonic acid production when its proper transport is impossible.

When, finally, in the process of neutralizing acid, the bicarbonates have been nearly used up, and the reaction is almost precisely at the neutral point, it is evident that protoplasm still possesses in the phosphates substances which have, thanks to the ionization constant of the ion  $\text{H}_2\text{PO}_4$  ( $2 \times 10^{-7}$ ), nearly the greatest efficiency which ordinary substances could possess in checking increase of acidity. Moreover the diffusibility of acid phosphates probably adds to their efficiency, much as the adjustment of carbonic acid tension does to that of the bicarbonates.

This conclusion is at least strongly indicated by the diffusion experiments of Maly<sup>24</sup> and by the experiments of Fitz, Alsberg, and Henderson<sup>25</sup> on the effect of feeding hydrochloric acid to rabbits. Another similar indication is the great predominance of acid phosphates in normal urine.

The above considerations show that in the matter of neutrality regulation the single phase equilibrium in the body is rendered far more efficient than it could otherwise be by the intervention of other phases acting selectively as reservoirs of supply and as vehicles of escape. Thus the theoretical considerations above developed regarding neutrality regulation must be modified for a heterogeneous system, in so far as relative efficiency of different substances is concerned; for, by the intervention of other phases, substances not possessing the highest efficiency in this process in a single phase system, may become enormously more efficient than any closed single phase system can be. Thus is the ideal single phase system easily surpassed by the heterogeneous system of the body.

We conclude, then, that the effectiveness of bicarbonates and phosphates to preserve neutrality in protoplasm is extraordinarily great. The bicarbonates constitute the first reserve, so to speak, in neutralizing acid. They are effective in a far greater degree than the salts of any acids in equal concentration could be because of the regulation of carbonic acid concentration by diffusion and excretion. The phosphates constitute the second reserve. When bicarbonates

<sup>24</sup> MALY: *Berichte der deutschen chemischen Gesellschaft*, 1876, ix, p. 164.

<sup>25</sup> FITZ, ALSBERG, and HENDERSON: *This journal*, 1907, xviii, p. 113.



are nearly used up, the efficiency of the phosphates becomes great, in fact probably greater than that of any substance not possessing the special advantage of removal of the acid ( $\text{KH}_2\text{PO}_4$  in this case) by diffusion. Only when an amount of acid equal to about three fourths of the total carbonic acid plus the total phosphoric acid of protoplasm has been added to protoplasm can there be a real beginning of acidity. The operation of other substances to check the growth of acidity is not here considered, but obviously serves to increase the efficiency of the process.

Growing alkalinity must also be checked by diffusion. In blood sodium bicarbonate is responsible for nearly 10 per cent of the total osmotic pressure, and a doubling of the blood alkalinity, which with constant carbonic acid tension can result only from doubling the concentration of sodium bicarbonate, must produce such a rise in osmotic pressure, about 10 per cent, that the kidneys will operate to lower it. This explains the failure of experimenters to produce more than a transitory increase in the alkalinity of blood as measured by titration.

It is evident that in general, wherever free carbonic acid exists, alkalinity of any real intensity is impossible, for by the action of the free acid all excess of alkali must be speedily and completely converted into bicarbonate; therefore in such a case the hydrogen ion concentration is defined by the equation  $(\text{H}) = k \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ , in which  $k$  varies with the temperature. For the temperatures occurring in living organisms the value of  $k$  can hardly be greater than  $10 \times 10^{-7}$  or less than  $1 \times 10^{-7}$ . Moreover, the concentration of bicarbonates in any living cell, no matter how differentiated its organization, probably can never approach nearly to the saturation point of such substance: at any rate, concentration is absolutely limited by that physical factor. Therefore in all living cells, of whatever sort, where carbonic acid is being constantly produced, unless a mechanism sufficiently powerful to produce free alkalis or carbonates from bicarbonates is present, alkalinity (concentration of hydroxyl ions) must unquestionably be very low indeed in the true aqueous solutions, and probably it is not in any case much greater than the alkalinity of mammalian blood.

For the future must be left the precise discussion of the rôle of other substances in neutrality regulation of the true aqueous solu-



tions of the body, as, for instance, the proteins,<sup>26</sup> and also the far more complicated and wholly dark problem of neutrality equilibrium within those complicated phases so characteristic of the organism, the colloidal hydrosols and hydrogels. It is certain, however, that, compared with the importance of the fundamental equilibria in the true solutions of the organism, all other substances are of secondary importance in the adjustment of the hydrogen and hydroxyl ionization. It is equally certain that whatever may be the case of the colloidal particles in this matter, they can influence the true solutions of the body only in those ways which have been carefully discussed in the course of this paper; their problem, then, is a separate one.<sup>27</sup>

#### CONCLUSIONS.

I. By analysis of the equilibrium between the four substances carbonic acid, sodium bicarbonate, mono-sodium phosphate, and disodium phosphate, it is shown, in accordance with the concentration law, that previous findings concerning the constitution of such systems are in accord with the theory. Curves are presented which define the equilibrium at 18°, and very accurately at 38°. The latter curve constitutes an accurate description of one of the fundamental equilibria within the typical cell of the warm-blooded animals.

II. It is pointed out that these studies prove that such systems possess nearly the highest efficiency which can occur in isolated aqueous solutions, for the preservation of neutrality.

III. It is shown by analyzing the intervention of other phases acting selectively as reservoirs of supply and as vehicles of escape for some or all of the above substances, that the efficiency of these

<sup>26</sup> It is, however, probable that in blood at 18° the sodium protein compounds are present in concentration less than one tenth that of sodium bicarbonate. Even if their concentration increases greatly as the temperature rises to 38°, a not improbable event in the light of the theory of carbonic acid excretion, they must still be present in much lower concentration than sodium bicarbonate, and accordingly they must possess at least a proportionately lower efficiency in preserving neutrality. Accordingly we may conclude that the importance of the proteins through their alkali-compounds in the preservation of neutrality in true solution is surely secondary. (See HENDERSON: *This journal*, 1908, xxi, p. 169.)

<sup>27</sup> It is evident that colloidal particles containing the amphoteric proteins may possess a high importance in neutrality regulation, both because of their power to combine with bases and acids, and because they may act selectively toward true aqueous solutions as reservoirs of supply and as vehicles of escape. It is planned to undertake in the near future the study of this interesting problem.

systems in the body is magnified to such a degree that they surpass the efficiency of any possible closed aqueous solutions of like concentration in preserving hydrogen ion concentration near  $0.3 \times 10^{-7}$  N. These considerations apply to both intra-cellular and extra-cellular aqueous solutions of the organism.

a. Increasing acidity is thus checked, not alone by reaction, operating to increase the acid components of the above solution, but also by their removal from the field of reaction. This is dependent probably in part upon their high diffusibility as acid substances, but also, at least in the case of carbonic acid, it is dependent upon the intervention of a complicated special mechanism existing for that purpose (the mechanism for the excretion of carbonic acid, including its transport in the blood, regulation of respiration, and selective activity of the lung epithelium). The case of acid phosphates is probably similar.

b. Increasing alkalinity is checked not alone by reaction, operating to increase the alkaline components of the above system, but also by their removal from the field of reaction. This is probably dependent, in part at least, upon simple diffusion, but also upon the fact that material increase in alkalinity must necessarily be accompanied by a large increase in osmotic pressure, which must be checked by the operation of the special mechanism existing for that purpose (excretion of more concentrated urine).

IV. It is shown, theoretically and experimentally, that blood and protoplasm probably become much more alkaline as the temperature rises, the hydrogen ion concentration remaining nearly constant, so that the alkalinity (concentration of hydroxyl ions) of blood in the body is probably about three times as great as it has been believed to be. Moreover, there is probably an increase of about one fourth in alkalinity when the normal body temperature rises to that of high fever. These relationships are due to the fact that the water constant ( $C = (\overset{+}{H}) \times (\overline{OH})$ ) increases much more rapidly with the temperature than do the ionization constants of carbonic acid, and of other weak acids which can take part in the equilibrium in the body.

V. These various conclusions, taken together, indicate that the physiological mechanism for the preservation of neutrality, or more precisely of a condition in which approximately  $(\overset{+}{H}) = 0.4 \times 10^{-7}$  N and  $(\overline{OH}) = 7.2 \times 10^{-7}$  N at  $38^{\circ}$  in the aqueous solutions

of the body, possesses a remarkable and unsuspected degree of efficiency. The regulation in true aqueous solution is due mainly to carbonates and phosphates, though other substances, as for instance the proteins, play a minor part. The efficiency is dependent upon the avidity of carbonic acid and mono-sodium phosphate as acids and on their high diffusibility, on heterogeneous equilibria selectively adjusted, and on the mechanism for the excretion of carbonic acid and for the regulation of osmotic pressure. The adjustment is of such a nature that large accidental variations in the concentrations of any of the constituents of the reaction, such as may normally occur, can hardly produce an appreciable effect upon the hydrogen and hydroxyl ion concentrations. That is to say, that the process possesses a high factor of safety,<sup>28</sup> thereby conforming to the requirements of an efficient biological process. It is interesting to note that we have herewith gained an intimate description of the chemical mechanism of such a process. Finally, it appears that pathological conditions or experimental assaults must cause death before general acidity of even very low intensity, or general alkalinity much above the normal, can be developed in the body as a whole, in accordance with clinical experience and experiment. Acidity cannot develop, because it must be preceded by an almost complete disappearance of carbonic acid from the blood, resulting in death. Alkalinity cannot develop because it must be preceded by a great rise in osmotic pressure, which must be checked by renal activity.

In this outline of the theory of neutrality regulation, the influence of the nature of the substances involved in the reactions, and the effects of temperature, pressure, and concentration and of selective physiological activity have been considered. In such a subject great precision is not demanded, because by the very nature of physiological processes variations and adaptability must always in certain respects prevent precision of definition. Accordingly, unless the present considerations shall prove to be in some manner fundamentally incorrect or fallacious, the description of the physiological regulation of neutrality here presented must be a true description of the general nature of the process, a frame within which all details of the process may be pictured. It is, in that case, open to quantitative changes, corrections, and amplifications, but qualitatively it must be correct.

<sup>28</sup> See MELTZER: *Journal of the American Medical Association*, Feb. 23, 1907, xlviii, p. 655.

## THE ACTION OF STRONTIUM COMPARED WITH THAT OF CALCIUM AND MAGNESIUM

BY S. J. MELTZER AND J. AUER.

[From the Department of Physiology and Pharmacology of the Rockefeller Institute  
for Medical Research.]

STRONTIUM belongs to the group of alkali earths and is closely related to calcium and magnesium. It is frequently assumed that within the body strontium exerts effects similar to those of the other two related elements. In previous papers on magnesium, and in the foregoing paper on the antagonism of calcium to magnesium, we described different profound effects which each of the two elements exercise upon the animal body. The question arose, which of the two does strontium resemble in its effect upon the living animal organism? In the experiments of Loeb, Zoethout, and others we find that strontium, like calcium, suppresses rhythmical as well as tonic contractions brought on by other substances. In the experiments of Overton we find, on the other hand, that, like calcium, strontium is capable of restoring the irritability of muscle, nerve trunk, and nerve endings, lost through the influence of some other chemical substance.

We do not intend to enter here upon the pharmacology of strontium. We made only a few short series of experiments with strontium to find an answer to the following three specific questions: (1) Is strontium capable of producing on mammals an anesthetizing and paralyzing effect similar to that produced by magnesium? (2) Is strontium capable of antagonizing the effects of magnesium in a way similar to that of calcium? (3) Is calcium capable of antagonizing strontium as it does magnesium?

We shall illustrate our results with one or two abbreviated protocols of experiments bearing upon each of these questions.

*Is strontium capable of producing anesthesia and paralysis in a*

*manner similar to that produced by magnesium?* — The experiments were made on rabbits, and strontium chloride was the salt used.

*Experiment 1.* — Gray male rabbit, 2090 gm.

10.30. A. M. Injected subcutaneously into the left flank 33 c.c.  $\text{SrCl}_2$  in  $m/1$  solution = 4 gm. per kilo.

11.15. Sitting up; respiration good, rapid; cannot be turned over on side.

12.15. Still able to sit up, but muscular weakness is marked; respiration fair, slow; moves body on light pinch of legs; lid reflex good.

3.00. Legs do not support body, stretch out sideways, especially front legs; able to move legs somewhat; feels pinch; respiration slow, shallow; lid reflex present.

4.00. Same. Able to raise head; shows tremor; turns over when placed on side.

9.30 (next morning). Dead; found in the same position occupied previous day, when last seen.

With magnesium, a dose of 1.75 gm. per kilo produces deep anesthesia and paralysis within thirty minutes. A slightly higher dose usually proves fatal within one hour. Here a dose of 4 gm. per kilo of the strontium salt did not affect the sensibility and did not abolish the reflexes. A very slowly developing paralysis was apparently the cause of the late death of the animal.

*Experiment 2 a.* — Gray female rabbit, 1680 gm.

1.50. Injected through ear vein 20 c.c.  $\text{SrCl}_2$  in  $m/8$  solution; no visible effect upon respiration.

2.00. Animal hops about on floor; no weakness of any kind.

2.15. Very lively. Injected again through ear vein, fairly rapidly, 24 c.c.  $\text{SrCl}_2$  in  $m/8$  solution.

Moderate weakness of legs, but sits up, moves around, cannot be turned over; respiration perhaps slower.

2.55. Sits quietly in one place; respiration slow. One hour later was apparently perfectly normal again.

*Experiment 2 b.* — Same animal, five days later.

12.00. Injected slowly through ear vein 6 c.c.  $\text{SrCl}_2$  in  $m/1$  solution. Respiration stopped, and lid reflex gone. After thirty seconds respiration began again, and lid reflex returned; feels pinch, lies on side, cannot sit up.

12.17. Sits up, can move about floor.

4.00. Seems perfectly well.

*Strontium Compared with Calcium and Magnesium.* 451

An intravenous injection of 44 c.c. of  $\text{SrCl}_2$   $m/8$  had practically no effect, and 6 c.c. in a molecular solution had only a momentary effect.

The effect of strontium has apparently no similarity with that of magnesium. No anesthesia whatsoever, and no loss of reflexes was noted. A very large dose (4 gm. per kilo) causes a very slowly developing paralysis.

*Is strontium capable of antagonizing the effects of magnesium in a way similar to the antagonistic action of calcium? —*

*Experiment 3.* — Yellow female rabbit, 1270 gm.

2.45. Injected subcutaneously, left side, 12 c.c.  $\text{MgCl}_2$   $m/1$  = 1.7 gm. per kilo.

3.10. Lying on side; respiration shallow; lid reflex active.

3.15. Respiration very shallow. Injected through ear nearly 15 c.c.  $m/8$   $\text{SrCl}_2$ . No improvement whatsoever; respiration gone, heart beats only occasionally. Hurriedly injected through ear vein "5 to 10" c.c.  $\text{CaCl}_2$  in  $m/8$  solution. Immediately after a short respiration; gradually began to breathe regularly and normally.

3.20. Unable to sit up.

Animal gradually recovered completely.

Strontium certainly did not neutralize the inhibitory effects of magnesium, but it did not hinder the neutralizing action of calcium.

*Experiment 4.* — Black and white rabbit, 1755 gm.

10.30. Injected subcutaneously left side 13 c.c.  $\text{MgCl}_2$  in  $m/1$  solution = 1.5 gm. per kilo. Slight massage.

10.50. Respiration shallow, slow; feels pinch; lies on side, limp; lid reflex active.

11.00. Injected into ear vein slowly 1 c.c.  $\text{SrCl}_2$  in  $m/1$  solution. Respiration apparently slightly deepened; lid reflex disappeared completely; no sensation whatsoever; no attempt at motion; animal more limp than before. Injected again through ear vein 5 c.c.  $m/8$   $\text{SrCl}_2$ . Respiration again slightly better, otherwise completely anesthetic and paralyzed. Remains in the same position for over two hours.

1.40. Suddenly turned over; feels pinch, but is very weak; remains on side when placed there.

5.00. Able to sit up; moves on pinching leg; lid reflex slight. From a dose of 1.5 gm.  $\text{MgCl}_2$  per kilo an animal usually recovers



fairly rapidly. When this rabbit received the intravenous injection of strontium, it had yet an active lid reflex, felt a pinch, and responded with a motion. Immediately on receiving some strontium, all this completely disappeared, and the animal remained much longer and much deeper in a state of coma and paralysis than it would have without the strontium. The respiration, however, improved very slightly after the injection of strontium.

Similar results were obtained in other experiments which were of a similar order. There is practically no similarity between the action of strontium and that of calcium in relation to the inhibitory effect of magnesium. Strontium not only does not neutralize, but seems to aggravate, some of the inhibitory symptoms. It seems, however, to cause a slight improvement of the respiration.

*Is calcium able to neutralize the effects of strontium? —*

*Experiment 5.* — Gray female, 2650 gm.

9.45. Injected subcutaneously in right flank 42 c.c.  $\text{SrCl}_2$  in  $m/1$  solution = 4 gm. per kilo.

12.20. Still able to sit up, legs under body.

1.50. Unable to hop, but legs not yet spreading, and animal is still in a fairly good condition. Injected 12 c.c.  $\text{CaCl}_2$   $m/8$  in ear vein. Condition became distinctly worse; cannot sit up any more, legs spreading sideways.

3.50 and 5.00 P. M. Unable to get legs under body; lying on belly with legs stretched out sideways; lid reflex active.

9.30 (next morning). Dead, lying in same position as left on previous day.

Calcium did not neutralize any of the effects of strontium; on the contrary, it seemed to have hastened the development of muscular weakness. Similar results were obtained in other experiments. Calcium, to say the least, does not antagonize the effects of strontium.

#### CONCLUSIONS.

The effects of strontium differ strikingly from those of magnesium; it does not cause anesthesia and does not affect sensibility and the reflexes. The paralysis which it causes develops very slowly and only after very large doses given subcutaneously.

The action of strontium differs completely from that of calcium, at least with respect to the neutralization of the inhibitory effect of



magnesium. It causes a very slight improvement of the respiration; otherwise it seems rather to aggravate and hasten the inhibitory symptoms due to magnesium, especially the paralysis.

Calcium is not antagonistic to the effects of strontium; if anything, it increases them.

As far as the effects are concerned with which we are dealing, strontium differs radically from magnesium as well as from calcium.

## SOME VASOMOTOR CHANGES IN THE CEREBRAL VESSELS OBTAINED BY STIMULATING THE CAROTID PLEXUSES.

By CARL J. WIGGERS.

[*From the Physiological Laboratory of the University of Michigan.*]

### I. INTRODUCTION.

**A**NATOMICAL arrangement foretells that the fibres regulating the cerebral vessels run either by way of the plexuses surrounding the internal carotid arteries or by those accompanying the vertebrals. From histological degeneration experiments<sup>1</sup> it appears probable that the fibres following the internal carotid route are postganglionic fibres with their cell bodies in the superior cervical ganglion. These fibres form more or less distinct bundles ascending on the inner aspect of the carotid arteries and separating into an inner and an outer division. Here they may often be seen by a lens as tiny strands which break up into plexuses. The outer division forms a plexus on the internal carotid artery while in the canal, and the inner gives rise to a similar plexus as that vessel comes to lie in the cavernous sinus. From these plexuses other perivascular networks are formed which supply the arterial branches from the Circle of Willis.

By the vertebral route postganglionic fibres pass from the inferior and upper thoracic ganglia through the vertebral canal with the artery, and ultimately form a plexus on the basilar artery. From this plexus filaments extend to its branches and seem to supply the more posterior portion of the brain.

To stimulate these plexuses and obtain results which should admit of no question as to whether these fibres have any control over the

<sup>1</sup> HUBER: *Journal of comparative neurology*, 1899, ix, p. 1.

cerebral vessels, has been the chief aim of my researches for the last three years. Up to the present time this desire has not been entirely realized, owing to the difficulty in stimulating these delicate filaments and in maintaining their functional activity while the vessels were being perfused. The results so far obtained, however, seem definite enough to warrant their immediate publication, although experimentation in this line will be continued.

## II. METHODS OF STIMULATING.

The brain vessels were perfused alone and the outflow continuously recorded by the methods described in my former papers.<sup>2</sup> To stimulate the nerve plexuses around the internal carotids, these arteries were first exposed in their bony canal somewhat beyond their internal ophthalmic branches. At first, attempts were made to dissect away the carotid sheaths and the nerve filaments from the arteries and to stimulate this strand. This method proved to be impracticable, for the nerve filaments were so injured during the manipulation that on stimulation they yielded no results. Recourse was then had to methods which left the nerve filaments intact. In some experiments the internal carotid arteries were ligated far down, and each branch tied off separately. The whole artery with the nerve plexus untouched was then placed on small insulated electrodes and stimulated. In later experiments one ligature was placed beyond the origin of the internal ophthalmic, and small insulated electrodes applied above this point. These electrodes were in every case applied to the artery and its plexus so as to remain absolutely stationary during the experiment. Thus no mechanical change in outflow due to their application could occur.

## III. RESULTS AND THEIR DISCUSSION.

*The Normal Flow.* — On perfusing the brain it was found in numerous experiments that the flow at first tended to increase gradually, later to decrease to and even below the original amount. The cause of the preliminary increase is due, in part to the more complete flushing out of the vessels by the perfusion fluid, but in part, also, to an active relaxation of the vessels under the influence of the warm solution. This factor can be demonstrated to exist, for, if a

<sup>2</sup> WIGGERS: This journal, 1905, xiv, p. 452; 1907, xx, p. 106.

cold solution is allowed to replace a warm one, a prompt decrease occurs. The subsequent decrease in flow seems to be the result of a gradually developing edema of the surrounding tissues.

The quantity of fluid perfused varies but little within short intervals of time, unless the position of the inflow cannula is interfered with or a vessel ruptures. If either of these accidents occurs, a *permanent* as well as sudden change in outflow results.

*Results on Stimulation.* — If the carotid sheaths are stimulated at a time when the physiological condition of the nerves and the technique are favorable, a decrease in flow through the brain vessels follows. The nature of this is shown in the segment of a curve reproduced in Fig. 1. Similar results were obtained in other experiments in each of which several stimulations were made. The data from these experiments are presented in Table I.

A glance at the outflow changes published in this table shows without question that stimulation of the plexuses surrounding the internal carotid arteries caused a decrease in flow through the cerebral vessels. The question naturally arises, Do these results also give any indication as to the most efficient form of stimulus for exciting these fibres, a form which could be most profitably used in further investigations? Conclusions on this point can be stated only provisionally, for not only are the data too few, but the condition of the nerves at the time of stimulation must have varied in different preparations. Few references in the literature have come to my notice as to the length of time after death of the animal that nerves may be expected to have an influence over perfused organs. Howell<sup>3</sup> states that the vagus action ceases one hour, and the accelerator action about four hours, after the death of the animal. Brodie and Dixon<sup>4</sup> found vaso-constrictor nerve trunks to be excitable as long as two to three hours after death. I have never obtained results on the cerebral vessels later than one and one-half hour after death. Between the time of decapitation and the final cessation of function there must be a period of gradually failing irritability, the influence of which on the degree of reaction cannot be determined. The results obtained offer some indication of the following relations between the reaction and the stimulus applied.

1. The strength of current employed seems to be one of the fac-

<sup>3</sup> HOWELL: *Journal of physiology*, 1906, xxxv, p. 134.

<sup>4</sup> BRODIE and DIXON: *Journal of physiology*, 1904, xxx, p. 499.

TABLE I.

Experiment No.	Time after de-capitation. min.	Stimulation.			Latent <sup>1</sup> period. sec.	Flow before stimulation. c.c.	Flow after stimulation. c.c.	Outflow difference. <sup>2</sup> per cent.	Degree of recovery. c.c.	Time for recovery. sec.
		Position of sec. coil	Dura-tion. in sec.	Rate. per sec.						
42 (1)	50	12	20	8	40	4.2 per 20 sec.	3.8 per 20 sec.	9.5	4.2 per 20 sec.	80
(2)	51½	12	36	8	60	4.4 per 20 sec.	3.7 per 20 sec.	15.8	4.4 per 20 sec.	80
(3)	53	17	20	8	20	4.4 per 20 sec.	3.2 per 20 sec.	27.2	3.9 per 20 sec.	160
(4)	55	12	40	32	80	3.9 per 20 sec.	3.5 per 20 sec.	10.3	4.2 per 20 sec.	180
(5)	57	17	25	32	80	3.9 per 20 sec.	3.5 per 20 sec.	10.3	4.4 per 20 sec.	180
62 (1)	72	10	85	16	30	4.2 per 30 sec.	3.2 per 30 sec.	23.8	3.9 per 30 sec.	50
(2)	77	10	120	16	60	3.7 per 30 sec.	2.9 per 30 sec.	21.6	4.2 per 30 sec.	30
65 (1)	60	10	100	32	20	2.4 per 30 sec.	1.8 per 30 sec.	25.0	2.4 per 30 sec.	45
(2)	64	10	70	32	10	2.7 per 30 sec.	2.2 per 30 sec.	18.5	3.0 per 30 sec.	90
(3)	100	12	40	16	65	3.2 per 30 sec.	2.5 per 30 sec.	21.8	2.7 per 30 sec.	120
(4)	103	12	60	16	50	4.4 per 30 sec.	3.7 per 30 sec.	15.9	4.4 per 30 sec.	200
74	84	10	35	32	5	5.8 per 20 sec.	5.1 per 20 sec.	12.0	5.6 per 20 sec.	80
76 (1)	45	12	32	32	10	8.3 per 30 sec.	6.7 per 30 sec.	19.2	8.1 per 30 sec.	60
(2)	47	12	32	32	5	7.6 per 30 sec.	7.2 per 30 sec.	5.2	7.6 per 30 sec.	25
(3)	49	12	40	32	10	7.6 per 30 sec.	7.0 per 30 sec.	7.8	7.9 per 30 sec.	80
84 (1)	?	14	40	16	15	2.4 per 20 sec.	1.9 per 20 sec.	20.8	2.4 per 20 sec.	80
(2)	?	14	70	16	15	2.5 per 20 sec.	1.9 per 20 sec.	24.0	2.2 per 20 sec.	70
(3)	?	18	16	32	5	2.2 per 20 sec.	1.6 per 20 sec.	27.2	2.3 per 20 sec.	45

<sup>1</sup> Latent period signifies interval elapsing between beginning of stimulation and the time a reaction occurs.<sup>2</sup> Percentage outflow difference =  $\frac{\text{Flow before stimulation} - \text{Flow after stimulation}}{\text{Flow before stimulation}} \times 100$ .

tors which determine the degree to which the vessels react (*cf.* Experiments 42<sup>2</sup> and 84<sup>3</sup> with other experiments).<sup>5</sup>

2. The duration of the stimulation is apparently a factor in the degree of reaction. *Stimulation with the same strength of current* gives more pronounced results in those cases where the duration is longest (*cf.* Experiments 74 and 65<sup>2</sup> with 65<sup>1</sup>, 62<sup>1</sup>, and 62<sup>2</sup>).

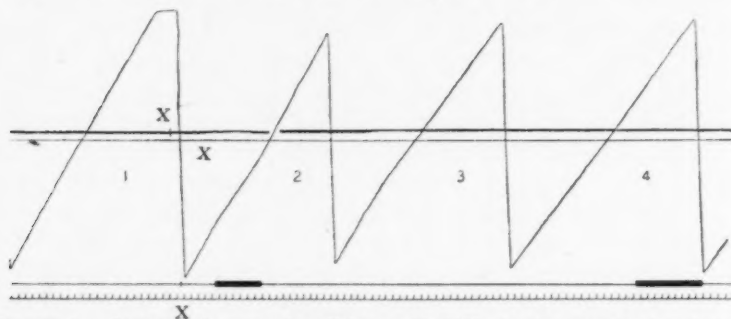


FIGURE 1.—Segment of curve taken February 20, 1908, showing records of perfusion pressure, outflow from cerebral vessels, time and duration of stimulation (secondary coil at 19, rate 32 per second) and time in seconds. Letters X indicate relative position of points. 1. Normal flow and perfusion pressure. 2. Effect of stimulating left carotid sheath on outflow and perfusion pressure. 3. Permanent mechanical effect on same, due to crushing nerves. 4. No effect on stimulation after crushing.

3. The rate of application of the stimulation has no noticeable effect on the degree of reaction, as shown by a comparison of experiments in which the *strength and duration of the stimulus* were the same but the rate altered (*cf.* Experiments 42<sup>1</sup> and 42<sup>2</sup> with 42<sup>4</sup>, 76<sup>1</sup>, and 76<sup>2</sup>).

4. The rate of application of the stimulus possibly influences the latent period of the reaction, which is generally shorter when a rapid rate is used (*cf.* Experiments 74, 76<sup>2</sup>, 84<sup>3</sup>, 65<sup>2</sup>, 76<sup>1</sup>, and 76<sup>3</sup> with 65<sup>3</sup>, 42<sup>2</sup>, and 62<sup>2</sup>).

The fact that the flow through the cerebral vessels diminishes on stimulation of the carotid plexuses certainly signifies that the vessels constricted. Does it necessarily signify that this was occasioned by vaso-constrictor fibres in these plexuses? Two other possibilities must first be answered. In the first place it may be suggested that the nerve fibres were not stimulated alone, but together with the

<sup>5</sup> Highest figures in column showing position of secondary coil indicate strongest current.

arterial wall. Any direct constriction of the tied off internal carotid artery must be entirely local. This would theoretically tend to increase slightly the amount of fluid in the Circle of Willis and thus augment the outflow and not decrease it. As a matter of fact, this local effect can never be seen to influence the outflow when nerve stimulation is unsuccessful, though in these cases the wall must have been stimulated.

In the second place, the possibility exists that the constriction of these vessels may have been caused, not through any nerve action, but by a spread of the current to them. Any one who is cognizant of the distance and obstacles existing between the point stimulated and the vessels which must react to cause a diminished flow cannot fail to see that a powerful spread would be necessary to affect them in this way. It was found that the usual method of excluding the spread of current by crushing the nerve beyond the point stimulated could only exceptionally be applied without causing outflow changes of such extent as to render the results valueless. Only in one experiment, a portion of which is shown in Fig. 1, was the proceeding successful. In this as in other experiments the current used was of such strength that, as previously tested, it caused no spread when tested on a nerve-muscle preparation under similar conditions, and ceased to give a depressor effect on a rabbit after crushing the nerve peripherally. In addition every precaution was taken to isolate with rubber dam all parts except that stimulated. The probability that a spread of current was the cause of the reaction is very small.

The conclusion may then be drawn that these results give direct physiological evidence of a nerve control over the cerebral vessels, — a conclusion which was foreshadowed by the reactions of vessels to drugs.



## THE EFFECT OF HÆMORRHAGE UPON THE VASOMOTOR REFLEXES.<sup>1</sup>

BY W. T. PORTER AND H. K. MARKS.

[*From the Laboratory of Comparative Physiology in the Harvard Medical School.*]

### I.

IT has long been known that the nerve cells are extraordinarily dependent upon oxygenated blood. When the blood supply is shut off by the closure of the cerebral and spinal vessels, the reflexes speedily disappear, and structural changes in the cells begin. If the anæmia be prolonged, even for minutes, recovery at best is difficult and slow. Important as are these conclusions, drawn from a complete or almost complete anæmia, they must yield in interest to the study of partial and progressive anæmia. Having once established the dependence of the nerve cells upon blood supply, we are concerned chiefly in determining upon how little food they can do their work. The present paper deals with progressive anæmia of the vasomotor cells from external hæmorrhage.

### II.

The influence of external hæmorrhage upon the vasomotor cells can be studied by measuring the reflex change in blood pressure occasioned by the stimulation of afferent nerves at the different levels to which the general blood pressure is reduced by the progressive loss of blood. This we have done, and we have also determined quantitatively the effect upon the reflex of raising the fallen pressure by the injection of defibrinated blood. Experiments in this general direction have been made before.<sup>2</sup> But as measure-

<sup>1</sup> The experiments upon which this paper is based were made in 1906 and briefly reported at the meeting of the American Physiological Society in December of that year. The experiments were repeated in 1907 and again in 1908.

<sup>2</sup> The literature has of late been frequently summarized. The most recent papers are those published in this journal by GUTHRIE, PIKE, and STEWART.

ments these earlier experiments are, in our opinion, insufficient for our present purpose in that they fail to apply the principle of percentile values. The absolute reflex change in blood pressure obtained at one level cannot be compared directly with that obtained at a different level. For example, in one series the stimulation of the sciatic nerve in the rabbit, while the blood pressure was 100 mm. Hg, caused a rise of 35 mm., and when the blood pressure was 50 mm., a stimulus of equal intensity still caused a rise of 35 mm. The absolute change was the same in both, but in the first instance this change was 35 per cent, while in the second it was 70 per cent. This is the well-known difference between the moral and the statistical value. An unfaithful trustee robs two women. One of these has \$40,000, the other \$20,000. From each he takes \$10,000. Their absolute loss is the same, but one woman can still live on her income, while the other must work or beg. It is necessary, then, in measuring vasomotor reflexes to take into account the level of the blood pressure at the beginning of stimulation, and this is done by expressing the change in blood pressure as a percentage of this level.<sup>3</sup>

The animals used were cats and rabbits. They were anæsthetized with ether and tracheotomized. Cannulas were placed in the central ends of the carotid arteries and in the external jugular or the crural vein. The vagus nerves were severed, and the sciatic and one or another of the brachial nerves prepared for stimulation. In the rabbit the depressor nerve was also prepared, and in the cat the central end of one severed vagus nerve was used as a depressor whenever preliminary stimulation showed the presence of a sufficient number of depressor fibres; sometimes these fibres ran apart from the vagus, as in the rabbit. Curare was cautiously injected into the vein and artificial respiration begun. The afferent nerves were now stimulated with induction currents to determine the normal reflex rise, or respectively fall of blood pressure. The normal reflex being recorded, the animal was bled from the carotid artery until the blood pressure fell to a new level. Here the reflex was again measured, and thus the condition of the vasomotor cells was tested at lower and lower levels, until at length the reflex failed. Then injections of normal saline solution, usually mixed with defibrinated blood, were made, and if the pressure rose, and

<sup>3</sup> W. T. PORTER: Vasomotor relations, the HARVEY lecture published in the Boston medical and surgical journal, 1908, clviii, p. 73.

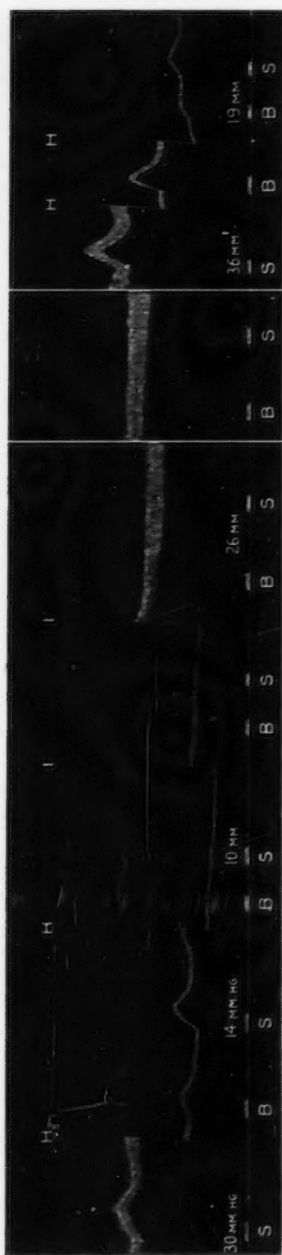


FIGURE 1.—Actual size. Experiment February 13, 1907. Curarized cat. Carotid blood pressure recorded with mercury manometer. The lower line marks the atmospheric pressure and the periods during which the central end of the divided brachial or sciatic nerve was stimulated with induction currents. The smoked paper moved 10 mm. per minute. At the beginning of the experiment (11.25 A.M.) the blood pressure was 140 mm. Hg. On giving curare, discontinuing the ether, and establishing artificial respiration, the blood pressure fell to 70 mm. On withdrawing first 25 c.c. and then 20 c.c. of blood, the pressure fell to 30 mm. Hg (11.53 A.M.); at this point Fig. 1 begins. At S the sciatic nerve and at B one of the brachial nerves was stimulated. At H blood was withdrawn, from left to right, 20, 12, 34, and 12 c.c. respectively. At I normal saline (0.9 per cent) mixed with defibrinated blood was injected, 10, 50, and 50 c.c. respectively. The hemorrhages reduced the blood pressure to 10 mm. Hg, but the reflex is still visible. After the low pressure had continued a few minutes, the nerve cells were so impaired that the reflex disappeared, although the blood pressure was raised to 30 mm. In about an hour, after generous injections of normal saline solution mixed with defibrinated blood, the reflex returned, showing that the bulbar cells had recovered, in part at least, from the almost total disability caused by their anæmia.

the reflex returned, the bleeding was repeated and the reflex again recorded as the pressure sank.

### III.

In studying the effect of hæmorrhage upon the vasomotor reflexes the investigator would wish to determine, first, the level at which the continued loss of blood would cause the disappearance of the reflexes, indicating that the vasomotor cells were no longer active. The answer to this question is given by Fig. 1. With the animal horizontal and all the experimental procedures well performed, it is probable that the arterial pressure may sink almost or quite to zero without the complete loss of the reflex.

Certainly in Fig. 1 the sciatic reflex is still visible at a pressure of 10 mm. Hg. If, however, the blood pressure remain even for a few moments at this low level, the recovery of the vasomotor cells will be difficult and at best very gradual, as in Fig. 1.

Not less interesting is the level at which the reflex begins to be seriously impaired by the failing nutrition of the vasomotor cells. The determination of this point is assisted by the statistical device described in a former paper.<sup>4</sup> As is there pointed out, the influence of "accidental" errors may be greatly diminished, even with the small number of observations collected in hæmodynamic studies, by dividing the observations into groups and taking the arithmetical mean of each group. The present observations have been thus treated. The rise following each stimulation of the sciatic or brachial nerve<sup>5</sup> was expressed as a percentage of the blood pressure at the beginning of stimulation, and the percentages taken between 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, and 71-80 mm. Hg blood pressure were added together. Each of the seven groups was now divided by the number of observations in the group. The observations in 1907 upon five cats and one rabbit are given in Table I. It appears that the reflex begins to fail when the arterial pressure has fallen to about 30 mm. Hg. It should, however, be especially noted that the percentile reflex rise remains more than half normal even below 20 mm.

<sup>4</sup> W. T. PORTER: This journal, 1907, xx, p. 401.

<sup>5</sup> The number of observations with depressor fibres was too small for statistical purposes.

## IV.

The persistence of a considerable reflex at such a low arterial pressure gives hope that the vasomotor cells will recover their full powers when the blood pressure is raised toward normal.

This hope is apparently realized in Fig. 1. The recovery of power, measured by the percentile reflex rise, is in this experiment

TABLE I.  
THE EFFECT OF HÆMORRHAGE UPON THE VASOMOTOR REFLEXES FOLLOWING  
STIMULATION OF THE SCIATIC AND THE BRACHIAL NERVES.

Blood pressure at the beginning of stimulation.	Number of observations.	Sum of individ- ual percentages.	Average per- centile rise in blood pressure.
mm. Hg			per cent
71-80	4	285	71
61-70	6	431	73
51-60	5	334	67
41-50	10	719	72
31-40	6	455	76
21-30	15	629	42
11-20	8	348	43

seemingly complete, but it will be remarked that the level to which the arterial pressure was restored by the injections employed was still much below the normal. At higher levels the recovery was not complete. Extended observations show how injurious are even brief anæmias.

Nevertheless, there emerges from these observations a clear impression that hæmorrhage may almost destroy the arterial pressure without much loss in the reflex power of the vasomotor cells, provided the animal be motionless in the horizontal position. The current in the bulbar capillaries ordinarily sets from the arteries toward the veins, but this hydrostatic custom is easily reversible. In animals such as the cat, when the arterial tone is sufficiently low, the stream will pass from the veins through the capillaries into the circle of Willis, — poor food, it is true, but better than none, and in fact quite able to maintain high vasomotor reflexes.

It is chiefly the force of gravity that impels the venous blood back through the capillaries. When arterial pressures are high, gravity may be neglected, — to make it negligible is, in truth, largely the purpose for which arterial tone exists, — but when the arterial pressure is low, gravity becomes a most important prophylactic and curative agent.

**CORRECTION.**

On page 177, line 5, the equation should read

$$(\dot{H}) = \frac{1 - MA}{MA} \cdot k \cdot 10^{-7}.$$



